INDIAN PHARMACOPOEIA 2022

Volume II

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Ministry of Health & Family Welfare

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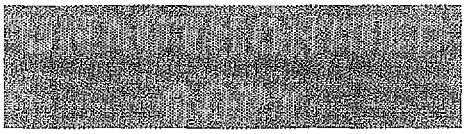

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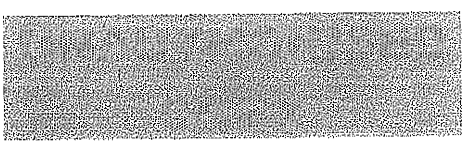

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GENERAL NOTICES

General Notices

General Statements

The General Notices provide the basic guidelines for the interpretation and application of the standards, tests, assays, and other specifications of the Indian Pharmacopoeia (IP), as well as to the statements made in the monographs and other texts of the Pharmacopoeia.

A monograph is to be constructed in accordance with any general monograph or notice or any appendix, note or other explanatory material that is contained in this Pharmacopoeia and that is applicable to that monograph. All statements contained in the monograph, except where a specific general notice indicates otherwise and with the exceptions given hereafter, constitute standards for the official articles. An article is not of pharmacopoeial quality unless it complies with all of the requirements stated.

Exceptions to the General Notices do exist, and where they do, the wording in the individual monograph or an appendix takes precedence and specifically indicates directions or the intent. Thus, the specific wording of standards, tests, assays and other specifications is binding wherever deviations from the General Notices exist. Likewise, where there is no specific mention to the contrary, the General Notices apply.

Name. The full name or title of this book, including addenda thereto, is Indian Pharmacopoeia 2022, abbreviated to IP 2022. In the texts, the term "Pharmacopoeia" or "IP" without qualification means the Indian Pharmacopoeia 2022 and any amendments and thereto.

Official and Official Articles. The word 'official' wherever used in this Pharmacopoeia or with reference thereto, is synonymous with 'pharmacopoeial', with 'IP' and with 'compendial'. The designation IP in conjunction with the official title on the label of an article is an indication that the article purports to comply with IP standards.

The following terms are used where the articles for which monographs are provided are to be distinguished.

An official substance is a single drug or a drug entity or a pharmaceutical aid for which the monograph title includes no indication of the nature of a dosage form.

An official preparation is a drug product (dosage form) and is the finished or partially finished preparation or product of one or more official substances formulated for use on the patient.

An article is an item for which a monograph is provided, whether an official substance or an official preparation.

Official Standards. The requirements stated in the monographs apply to articles that are intended for medicinal use but not necessarily to articles that may be sold under the same name for other purposes.

An article is not of Pharmacopoeial quality unless it complies with all the requirements stated in the monograph. This does not imply that performance of all the tests in a monograph is necessarily a prerequisite for a manufacturer in assessing compliance with the Pharmacopoeia before release of a product.

Pharmacopoeial requirements for articles used in veterinary medicine are established on the same basis as those used in human medicine. It should be noted that no requirement in the pharmacopoeia can be taken in isolation. A valid interpretation of any particular requirement depends upon it being read in context of the monograph as a whole, the specified method of analysis, the relevant General Notices and where appropriate the General Monographs.

Where a preparation that is the subject of a monograph in the Indian Pharmacopoeia is supplied for use in veterinary medicine, the standards of Indian Pharmacopoeia apply unless otherwise justified and authorized.

The active pharmaceutical ingredients (drug substances), excipients (pharmaceutical aids), pharmaceutical preparations (dosage forms) and other articles described in the monographs are intended for human and veterinary use (unless explicitly restricted to one of these uses). It may be noted, however, that in the event of doubt of interpretation in any text of Veterinary monographs of IP, Indian Pharmacopoeia Commission (IPC) should be consulted.

The requirements given in the monographs are not framed to provide against all possible impurities, contaminants or adulterants; they provide appropriate limitation of potential impurities only.

A preparation must comply with the requirements specified, throughout its shelf-life assigned to it by the manufacturer. For opened or broached containers, the maximum period of validity for use will be as may be stated in the individual monograph. Nevertheless, the responsibility for assigning the period of validity shall be with the manufacturer.

Added Substances. An official substance, as distinguished from an official preparation, contains no added substances except when specifically permitted in the individual monograph. Unless otherwise specified in the individual monograph, or elsewhere in the General Notices, suitable substances may be added to an official preparation to enhance its stability, preserve its properties, usefulness or elegance, or to facilitate its preparation. Such auxiliary substances shall be harmless in the amounts used, shall not exceed the minimum quantity required to provide their intended effect, shall not impair the therapeutic efficacy or the bioavailability or safety of the preparation and shall not interfere with any of the tests and assays prescribed for determining compliance with the official

standards. Particular care should be taken to ensure that such substances are free from harmful organisms. The freedom to the manufacturers to add auxiliary substances imposes on them the responsibility of satisfying the licensing authorities on the purpose of the addition and the innocuity of such substances. No substance shall be added to conceal any defect or damage or deficiency in the substance or formulation.

Alternative Methods. The tests and assays described are the official methods upon which the standards of the Pharmacopoeia are based. Alternative methods of analysis may be used for control purposes, provided that the methods used are shown to give results of equivalent accuracy and enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official methods were used. Automated procedures utilising the same basic chemistry as the test procedures given in the monograph may also be used to determine compliance. Such alternative or automated procedures must be validated and are subject to approval by the authority competent to authorised manufacturer of substance or product.

In the event of doubt or dispute, the methods of analysis of the Pharmacopoeia are alone authoritative and only the result obtained by the procedure given in this Pharmacopoeia is conclusive.

Meanings of Terms

Alcohol. The term "alcohol" without qualification means ethanol (95 per cent). Other dilutions of ethanol are indicated by the term "ethanol" or "alcohol" followed by a statement of the percentage by volume of ethanol (C_2H_6O) required.

Desiccator. A tightly-closed container of suitable size and design that maintains an atmosphere of low moisture content by means of silica gel or phosphorus pentoxide or other suitable desiccant.

Drying and ignition to constant weight. Two consecutive weighings after the drying or igniting operations do not differ by more than 0.5 mg, the second weighing following an additional period of drying or of ignition respectively appropriate to the nature and quantity of the residue.

Ethanol. The term "ethanol" without qualification means anhydrous ethanol or absolute alcohol.

Filtration. Unless otherwise stated, filtration is the passing of a liquid through a suitable filter paper or equivalent device until the filtrate is clear.

Freshly prepared. Made not more than 24 hours before it is used.

Label. Any printed packing material, including package inserts that provide information on the article.

Negligible. A quantity not exceeding 0.50 mg.

Solution. Where the name of the solvent is not stated, "solution" implies a solution in water. The water used complies with the requirements of the monograph on Purified Water.

Temperature. The symbol ° used without qualification indicates the use of the Celsius thermometric scale.

Water. If the term is used without qualification it means Purified Water of the Pharmacopoeia. The term 'distilled water' indicates Purified Water prepared by distillation.

Water-bath. A bath of boiling water unless water at another temperature is indicated. Other methods of heating may be used provided the required temperature is approximately maintained but not exceeded.

Provisions Applicable To Monographs and Test Methods

Expression of Contents. Where the content of a substance is defined, the expression "per cent" is used according to circumstances with one of two meanings:

— per cent w/w (percentage, weight in weight) expressing the number of grams of substance in 100 grams of final product,

— per cent v/v (percentage, volume in volume) expressing the number of millilitres of substance in 100 millilitres of final product.

The expression "parts per million" refers to the weight in weight, unless otherwise stated.

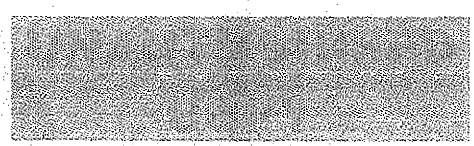
Where the content of a substance is expressed in terms of the chemical formula for that substance an upper limit exceeding 100 per cent may be stated. Such an upper limit applies to the result of the assay calculated in terms of the equivalent content of the specified chemical formula. For example, the statement 'contains not less than 99.0 per cent and not more than 101.0 per cent of $C_7H_6O_2$ implies that the result of the assay is not less than 99.0 per cent and not more than 101.0 per cent, calculated in terms of the equivalent content of $C_7H_6O_2$.

Where the result of an assay or test is required to be calculated with reference to the dried, anhydrous, ignited substance, or the substance free from solvent, the determination of loss on drying, water content, loss on ignition, content of the specified solvent, respectively is carried out by the method prescribed in the relevant test in the monograph.

Expression of Concentrations. The following expressions in addition to the ones given under Expression of Content are also used:

— per cent w/v (percentage, weight in volume) expressing the number of grams of substance in 100 millilitres of product,

— per cent v/w (percentage, volume in weight) expressing the number of millilitres of substance in 100 grams of product.



Usually, the strength of solutions of solids in liquids is expressed as percentage weight in volume, of liquids in liquids as percentage volume in volume, of solids in semi-solid bases (e.g. creams) and of gases in liquids as percentage weight in weight.

When the concentration of a solution is expressed as parts of dissolved substance in parts of solution, it means parts by weight (g) of a solid in parts by volume (ml) of the final solution; as parts by weight (g) of a gas in parts by weight (g) of the final solution.

When the concentration of a solution is expressed in molarity designated by the symbol M preceded by a number, it denotes the number of moles of the stated solute contained in sufficient Purified Water (unless otherwise stated) to produce 1 litre of solution.

Abbreviated Statements. Incomplete sentences are employed in parts of the monographs for directness and brevity (for example, Iodine Value. Not more than ……; Relative Density. …….to……..) Where the tests are abbreviated, it is to be understood that the test method referred to in brackets provides the method to be followed and that the values specified are the applicable limits.

Weights and Measures. The metric system of weights and measures is employed in the Pharmacopoeia. All measures are required to be graduated at 25° and all measurements in tests and assays, unless otherwise stated, are to be made at that temperature. Graduated glass apparatus used in analytical operations shall comply with the requirements stated in Chapter 2.1.6.

Monographs

General Monographs

General monographs on dosage forms include requirements of general application and apply to all preparations within the scope of the Introduction section of the general monograph, except where a preamble limits the application. The requirements are not necessarily comprehensive for a given specific preparation; additional requirements may sometimes be given in the individual monograph for it.

Production. Statements given under the heading Production relate to particular aspects of the manufacturing process and are not necessarily comprehensive. However, they are mandatory instructions to manufacturers. They may relate, for example, to source materials, to the manufacturing process and its validation and control, to any in-process testing that is to be carried out by the manufacturer on the final product either on selected batches or on each batch prior to release. All this cannot be verified on a sample of the final product by an independent analyst. It is for the licensing authority to verify that the instructions have been followed.

The absence of a section on Production does not imply that attention to features such as those given above is not required. An article described in a monograph of the Pharmacopoeia is to be manufactured in accordance with the principles of good manufacturing practice and in accordance with the requirements of the Drugs and Cosmetics Rules, 1945. The general principles applicable to the manufacture and quality assurance of drugs and preparations meant for human use apply equally to veterinary products as well.

Manufacture of Drug Products. The opening definitive statement in certain monographs for drug products is given in terms of the active ingredient(s) only. Any ingredient(s) other than those included in the statement, must comply with the general notice on Excipients and the product must conform to the Pharmacopoeial requirements.

Official preparations are prepared only from ingredients that comply with the requirements of the pharmacopoeial monographs for those individual ingredients for which monographs are provided.

Excipients. Any substance added in preparing an official preparation shall be innocuous, shall have no adverse influence in the therapeutic efficacy of the active ingredients and shall not interfere with the tests and assays of the Pharmacopoeia. Care should be taken to ensure that such substances are free from harmful organisms.

Individual Monographs

Drug products that are the subject of an individual monograph are also required to comply with the tests given in the general monographs.

Titles. The main title for a drug substance is the International Non-proprietary Name (INN) approved by the World Health Organization. Subsidiary names and synonyms have also been given in some cases; where included, they have the same significance as the main title.

The main titles of drug products are the ones commonly recognised in practice. Synonyms drawn from the full non-proprietary name of the active ingredient or ingredients have also been given. Where, however, a product contains one or the other of different salts of an active molecule, the main title is based on the full name of the active ingredient. For example, Chloroquine Phosphate Tablets and Chloroquine Sulphate Tablets.

Chemical Formulae. When the chemical structure of an official substance is known or generally accepted, the graphic and molecular formulae are normally given at the beginning of the monograph for information. This information refers to the chemically pure substance and is not to be regarded as an indication of the purity of the official material. Elsewhere, in statement of purity and strength and in descriptions of

processes of assay, it will be evident from the context that the formulae denote the chemically pure substances.

Where the absolute stereochemical configuration is specified, the International Union of Pure and Applied Chemistry (IUPAC) *R/S* and *E/Z* systems of designation have been used. If the substance is an enantiomer of unknown absolute stereochemistry, the sign of the optical rotation, as determined in the solvent and under the conditions specified in the monograph, has been attached to the systematic name. An indication of sign of rotation has also been given where this is incorporated in a trivial name that appears on an IUPAC preferred list.

Atomic and Molecular Weights. The atomic weight or molecular weight is shown, as and when appropriate at the top right hand corner of the monograph. The atomic and molecular weights and graphic formulae do not constitute analytical standards for the substances described.

Definition. The opening statement of a monograph is one that constitutes an official definition of the substance, preparation or other article that is the subject of the monograph. In certain monographs for pharmaceutical preparations the statement is given in terms of the principal ingredient(s).

In monographs on vegetable drugs, the definition indicates whether the subject of the monograph is, for example, the whole drug or the drug in powdered form.

Certain pharmaceutical substances and other articles are defined by reference to a particular method of manufacture. A statement that a substance or article is prepared or obtained by a certain method constitutes part of the official definition and implies that other methods are not permitted. A statement that a substance may be prepared or obtained by a certain method, however, indicates that this is one possible method and does not imply that other methods are not permissible.

Statement of content. The limits of content stated are those determined by the method described under Assay.

Category. The statement of category is provided for general information only and is indicative of the medical or pharmaceutical basis for recognition in the Pharmacopoeia. It generally represents an application of the best known pharmacological action of the article or of its active ingredient. The statement under the heading 'Category' are also subject to regulations under the D&C Act 1940 and rules theirunder. In the case of pharmaceutical aids it may indicate the more common usage of the article. The statement is not intended to limit in any way the choice or use of the article nor to indicate that it has no other activity or use.

Usual strength. The statement on the usual strength(s) of a preparation given in the individual monograph indicates the strength(s) usually marketed for information of the pharmacist and the medical practitioner. It does not imply that a strength other than the one(s) mentioned in the individual monograph meeting all the prescribed requirements cannot be manufactured and marketed with the approval of the appropriate authority.

Description. The statements under the heading Description are not to be interpreted in a strict sense and are not to be regarded as official requirements.

Solubility. Statements on solubility are given in Chapter 2.4.26 and are intended as information on the approximate solubility at a temperature between 15° and 30°, unless otherwise stated, and are not to be considered as official requirements. However, a test for solubility stated in a monograph constitutes part of the standards for the substance that is the subject of that monograph.

Residual solvents. The requirements, guidance and information on residual solvents for pharmaceutical use are given in the chapter entitled Residual Solvents (5.4).

All IP articles are subject to relevant control of residual solvents, even when no test is specified in the individual monograph. If solvents are used during production, they must be of suitable quality. In addition, the toxicity and residual level of each solvent shall be taken into consideration and the solvents limited according to the principles defined and the requirements specified in Chapter 5.4. Residual Solvent, using the general methods presented therein or other suitable methods.

Test Methods

References to general methods of testing are indicated by test method numbers in brackets immediately after the heading of the test or at the end of the text.

Identification. The tests given under the heading Identification are not necessarily sufficient to establish absolute proof of identity. They provide a means of verifying that the identity of the material under examination is in accordance with the label on the container.

In certain monographs alternative series of identification tests are given; compliance with either one or the other set of tests is adequate to verify the identity of the article.

When tests for infrared absorption are applied to material extracted from formulated preparations, strict concordance with the specified reference spectrum may not always be possible, but nevertheless a close resemblance between the spectrum of the extracted material and the specified reference spectrum should be achieved.

Tests and Assays

The tests and assays are the official methods upon which the standards of the Pharmacopoeia depend. The requirements are not framed to take into account all possible impurities. It is

not to be presumed, for example, that an impurity that is not detectable by means of the prescribed tests is tolerated. Material found to contain such an impurity is not of pharmacopoeial quality if the nature or amount of the impurity found is incompatible with good pharmaceutical practice.

Pharmacopoeial methods and limits should be used merely as compliance requirements and not as requirements to guarantee total quality assurance. Tests and assays are prescribed for the minimum sample available on which the attributes of the article should be measured. Assurance of quality must be ensured by the manufacturer by the use of statistically valid sampling and testing programmes.

Tests. Unless otherwise stated, the assays and tests are carried out at a temperature between 20° and 30°.

Where it is directed that an analytical operation is to be carried out 'in subdued light', precautions should be taken to avoid exposure to direct sunlight or other strong light. Where a procedure is directed to be performed 'protected from light' precautions should be taken to exclude actinic light by the use of low-actinic glassware, working in a dark room or similar procedures.

For preparations other than those of fixed strength, the quantity to be taken for a test or an assay is usually expressed in terms of the active ingredient. This means that the quantity of the active ingredient expected to be present and the quantity of the preparation to be taken are calculated from the strength stated on the label.

Other Tests. In the monographs on dosage forms and certain preparations, under the sub-heading 'Other tests' it is stated that the article complies with the tests stated under the general monograph of the relevant dosage form or preparation. Details of such tests are provided in the general monographs.

Limits. The limits given are based on data obtained in normal analytical practice. They take into account normal analytical errors, of acceptable variations in manufacture and of deterioration to an extent that is acceptable. No further tolerances are to be applied to the limits for determining whether or not the article under examination complies with the requirements of the monograph.

Quantities. Unless otherwise stated, the quantities to be taken for assays, limit tests and other tests are of the substance under examination.

In tests with numerical limits and assays, the quantity stated to be taken for testing is approximate. The amount actually used, which may deviate by not more than 10 per cent from that stated, is accurately weighed or measured and the result of analysis is calculated from this exact quantity. In tests where the limit is not numerical but usually depends upon comparison with the behaviour of a reference in the same conditions, the stated quantity is taken for testing. Reagents are used in the prescribed amounts.

Quantities are weighed or measured with an accuracy commensurate with the indicated degree of precision. For weighings, the precision is plus or minus 5 units after the last figure stated. For example, 0.25 g is to be interpreted as 0.245 g to 0.255 g. For the measurement of volumes, if the figure after the decimal point is a zero or ends in a zero, e.g. 10.0 ml or 0.50 ml, the volume is measured using a pipette, a volumetric flask or a burette, as appropriate; in other cases, a graduated measuring cylinder or a graduated pipette may be used. Volumes stated in microlitres are measured using a micropipette or microsyringe.

The term 'transfer' is used generally to indicate a quantitative operation.

Apparatus. Measuring and weighing devices and other apparatus are described in the chapter entitled 'Apparatus for Tests and Assays'. A specification for a definite size or type of container or apparatus in a test or assay is given merely as a recommendation.

Unless otherwise stated, comparative tests are carried out using identical tubes of colourless, transparent, neutral glass with a flat base, commonly known as Nessler cylinders.

Reagents and Solutions. The reagents required for the tests and assays of the Pharmacopoeia are defined in the various chapters showing their nature, degree of purity and the strengths of the solutions to be made from them. The requirements set out are not intended to imply that the materials are suitable for use in the test concerned; reagents not covered by monographs in the pharmacopoeia shall not be claimed to be of IP quality.

The term 'analytical reagent grade of commerce' implies that the chemical is of a high degree of purity wherein the limits of various impurities are known. Where it is directed to use a 'general laboratory reagent grade of commerce' it is intended that a chemically pure grade material, not necessarily required to be tested for limiting or absence of certain impurities, is to be used.

Indicators. Where the use of an indicator solution is mentioned in an assay or test, approximately 0.1 ml of the solution shall be added, unless otherwise directed.

Reference Substances. Certain monographs require the use of a chemical reference substance or a biological reference preparation or a reference spectrum These are authentic specimens chosen and verified on the basis of their suitability for intended use as prescribed in the Pharmacopoeia and are not necessarily suitable in other circumstances.

IP Reference Substances, abbreviated to IPRS are issued by the Indian Pharmacopoeia Commission (IPC). They are the official standards to be used in cases of arbitration.

Biological Reference Substances, also abbreviated to IPRS and Standard Preparations of antibiotics are issued by

agencies authorised by the IPC. They are standardized against the International Standards and Reference Preparations established by the World Health Organization (WHO). The potency of these preparations is expressed in International Units.

Reference spectra are published by the IPC and they are accompanied by information concerning the conditions used for sample preparation and recording of the spectra.

Test Animals. The animal experiments are carried out in accordance with the provisions of 'The Prevention of Cruelty to Animals Act, 1960' and 'CPCSEA Guidelines' so as to prevent the infliction of unnecessary pain, suffering and prevention of cruelty to animals. Unless otherwise directed, animals used in a test or an assay shall be healthy and are drawn from a uniform stock, and have not previously been treated with any material that will interfere with the test or the assay.

Rounding Rules for Calculation of Results. The observed or calculated values should be rounded off to the number of decimal places that is in agreement with the limit expression. Numbers should not be rounded up or down until the final calculations for the reportable value have been completed. Intermediate calculations (e.g., slope for linearity) may be rounded for reporting purposes, but the original (not rounded) value should be used for any additional required calculations. Acceptance criteria are fixed numbers and are not rounded.

When rounding is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is 4 or smaller, it is eliminated and the preceding digit is left unchanged. If this digit is 5 to 9, it is eliminated and the preceding digit is increased by 1.

Table 1 – Illustration of Rounding Numerical values for comparison with Requirements

Pharmacopoeial Requirement	Unrounded Value (per cent)	Rounded Result (per cent)	Conforms
Assay limit	97.96	98.0	Yes
≥ 98.0 per cent	97.92	97.9	No
	97.95	98.0	Yes
Assay limit	101.55	101.6	No
≤ 101.5 per cent	101.46	101.5	Yes
	101.45	101.5	Yes
Limit test	0.025	0.03	No
≤ 0.02 per cent	0.015	0.02	Yes
	0.027	0.03	No
Limit test ≤ 3 ppm	3.5 ppm	4 ppm	No
	3.4 ppm	3 ppm	Yes
	2.5 ppm	3 ppm	Yes

Storage. Statements under the side-heading 'Storage' constitute non-mandatory advice. The articles of the Pharmacopoeia are to be stored under conditions that prevent contamination and, as far as possible, deterioration. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat and light are indicated, where appropriate, in the individual monograph.

Specific directions are given in some monographs with respect to the temperatures at which Pharmacopoeial articles should be stored, where it is considered that usage at a lower or higher temperature may produce undesirable results. The storage conditions are defined by the following terms:

- Store in a dry, well-ventilated place at a temperature not exceeding 30°
- Store in a refrigerator (2° to 8°). Do not freeze
- Store in a freezer (-2° to -18°)
- Store in a deep freezer (Below -18°)

Storage conditions not related to temperature are indicated in the following terms:

- Store protected from light
- Store protected from light and moisture

Where no specific storage directions or limitations are given in the monograph or in the D&C rules 1945 or by the manufacturer, it is to be understood that the storage conditions include protection from moisture, direct sunlight, freezing and excessive heat (any temperature above 40°).

Storage Containers. The requirements, guidance and information on containers for pharmaceutical use are given in the chapter entitled Containers (6.2).

In general, an article should be packed in a well-closed container i.e. one that protects the contents from contamination by extraneous solids, liquids, moisture or vapours and from loss of the article under normal conditions of handling and storage and preserves the properties of the drug. Containers, unless otherwise specified, or of the nature such as capsule shall, foils of strips etc, shall allow examination of the contents inside. Closures used shall also of suitable properties and quality to protect the drug from any contamination and shall not be the source of contamination by themselves. Notices as may be needed in respect of Radiopharmaceuticals may also be incorporated.

Where, additionally, loss or deterioration of the article from effervescence, deliquescence or evaporation under normal conditions of storage is likely, the container must be capable of being tightly closed, and re-closed after use.

In certain cases, special requirements of pack have been indicated in some monographs under Storage, using expressions that have been defined in chapter 6.2.

Labelling. The labelling of drugs and pharmaceuticals is governed by the Drugs and Cosmetics Rules, 1945. The statements that are given in the monographs under the side-heading 'Labelling' are not comprehensive. Only those that are necessary to demonstrate compliance or otherwise with the monograph have been given and they are mandatory. For example, in the monograph on Betamethasone Sodium Tablets the labelling statement is "The label states the strength in terms of the equivalent amount of betamethasone".

ACTIVE PHARMACEUTICAL INGREDIENTS AND DOSAGE FORMS

General Requirements

The Pharmacopoeia provides monographs of active pharmaceutical ingredients (that are used as active substances or excipients) and dosage forms (consisting of active substances that may be combined with excipients). Additionally, the general requirements including the processes for the preparation of many of them and the tests of a general nature applicable to article described in the monograph are given in the following pages. In addition to define the active pharmaceutical ingredients and dosage forms, this section presents the general principles involved in the production of some of them.

The requirements for compliance with tests given under active pharmaceutical ingredients are indicated in each monograph of the pharmaceutical substances. All active pharmaceutical ingredients comply with the requirements of general monograph, unless otherwise indicated.

The requirements for compliance with the tests given under each dosage form are indicated in each monograph of a drug product under the heading 'Other tests'. These tests are mandatory and are additional to the tests given in the individual monograph.

Active Pharmaceutical Ingredients

Pharmaceutical Substances

Active Pharmaceutical Ingredients (APIs) are any organic or inorganic substances that are used as active substances or excipients for the production of medicinal products for human or veterinary use. They may be obtained from natural sources or produced by extraction from raw materials, fermentation or synthesis.

This general monograph does not apply to biological and biotechnological products, oligonucleotides, products of fermentation and semi-synthetic products derived there from, to crude products of animal or plant origin or herbal products.

Active Pharmaceutical Ingredients may be used as such or as starting materials for subsequent formulation to prepare medicinal products. Depending on the formulation, certain substances may be used either as active substances or as excipients. Solid substances may be compacted, coated, granulated, powdered to certain fineness, or processed in other ways. A monograph is applicable to a substance processed with an excipient only where such processing is mentioned in the monograph.

Polymorphism. Individual monographs do not usually specify crystalline or amorphous forms, unless bioavailability is affected. All forms of an active substance comply with the requirements of the monograph, unless otherwise indicated. Where a substance may show polymorphism, this may be stated under description in order to draw this to the attention of the user who may have to take this characteristic into consideration during formulation of a preparation.

Production

Active Pharmaceutical Ingredients are manufactured by procedures that are designed to ensure a consistent quality and comply with the requirements of the individual monograph or approved specification. The manufacture of active substances must take place under conditions of good manufacturing practice.

The provisions of chapter 5.5.Impurities, apply to the control of impurities in active pharmaceutical ingredients. If solvents are used during production, they are of suitable quality. In addition, their toxicity and their residual level are taken into consideration as mentioned in chapter 5.4. Residual Solvents. If water is used during production, it is of suitable quality. The identity of elemental impurities derived from intentionally added catalysts and reagents is known, and strategies for controlling them should be established.

If substances are produced or processed to yield a certain form or grade, that specific form or grade of the substance complies with the requirements of the monograph. Certain functionality-related tests may be described to control properties that may influence the suitability of the substance and subsequently the properties of dosage forms prepared from it.

Powdered substances. These may be processed to obtain a certain degree of fineness.

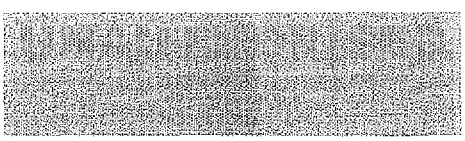
Compacted substances. These are processed to increase the particle size or to obtain particles of a specific form and/or to obtain a substance with a higher bulk density.

Coated active substances. These consist of particles of the active substance coated with one or more suitable excipients.

Granulated active substances. These are particles of a specified size and/or form produced from the active substance by granulation directly or with one or more suitable excipients.

If substances are processed with excipients, these excipients comply with the requirements of the relevant monograph or, where no such monograph exists, the specifications approved by regulatory authority shall comply.

Where active substances have been processed with excipients to produce, for example, coated or granulated substances, the processing is carried out under conditions of good manufacturing practice and the processed substances are regarded as intermediates in the manufacture of a medicinal product.



Description. The statements under the heading Description are not to be interpreted in a strict sense and are not to be regarded as official requirements. They are given for information.

Solubility. The statements on solubility are given under chapter 2.4.26 and are intended as information on the approximate solubility at a temperature between 15° and 30°, unless otherwise stated, and are not to be considered as official requirements. However, a test for solubility stated in a monograph constitutes part of the standards for the substance that is the subject of that monograph.

Identification

Where under Identification an individual monograph contains alternative series of identification tests; compliance with either one or the other set of tests is adequate to verify the identity of the article.

Certain monographs contain a cross-reference to a test prescribed in the Tests section of the monograph. It may be used to simplify the work of the analyst carrying out the identification and the prescribed tests.

Tests

Polymorphism. If the nature of a crystalline or amorphous form imposes restrictions on its use in preparations, the nature of the specific crystalline or amorphous form is identified, its morphology is adequately controlled and its identity is stated on the label as mentioned in chapter 2.5.11.

Related substances. Unless otherwise prescribed or justified and authorized, organic impurities in active substances are to be reported, identified wherever possible, and qualified as indicated in chapter 5.5. Impurities. Specific thresholds may be applied for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects.

For DNA reactive impurities, the requirements of ICH Guideline- M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk must be complied with for active substances to be used in medicinal products for human use, in cases defined in the scope of the guideline.

If the individual monograph does not provide suitable control for a new impurity, a suitable test for control must be developed and included in the specification for the substance.

Heavy metals. Unless otherwise prescribed or justified and authorized, heavy metal tests apply to the active pharmaceutical ingredients as mentioned in chapter 2.3.13.

OR

Elemental impurities. Permitted daily exposures for elemental impurities as mentioned in chapter 5.10 apply to the medicinal product. Individual monographs on active pharmaceutical ingredients do not contain specifications for elemental impurities unless otherwise prescribed.

Residual solvents. The residual solvents are limited according to the principles defined in chapter 5.4, using specified general method or another suitable method after validation. Where a quantitative determination of a residual solvent is carried out and a test for loss on drying is not carried out, the content of residual solvent is taken into account for calculation of the assay content of the substance, the specific optical rotation and the specific absorbance.

Microbiological quality. Individual monographs give acceptance criteria for microbiological quality wherever such control is necessary. Table 5– Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use, under chapter 2.2.9. Microbial Contamination in Nonsterile products, gives recommendations on microbiological quality that are of general relevance for substances subject to microbial contamination. Depending on the nature of the substance and its intended use, different acceptance criteria may be justified.

Sterility. If intended for use in the manufacture of sterile dosage forms without a further appropriate sterilization procedure, or if offered as sterile grade, the active pharmaceutical ingredients complies with the test for sterility as mentioned in chapter 2.2.11.

Bacterial endotoxins. The active pharmaceutical ingredients complies with the test for bacterial endotoxins if it is labelled as a bacterial endotoxin-free grade or if it is intended for use in the manufacture of parenteral preparations or preparations for irrigation without a further appropriate procedure for the removal of bacterial endotoxins. The limit, when not indicated in the individual monograph, is determined in accordance with chapter 2.2.3.

Pyrogens. If the test for pyrogens is justified rather than the test for bacterial endotoxins and if a pyrogen-free grade is offered, the active pharmaceutical ingredients complies with the test for pyrogens in accordance with recommendations of chapter 2.2.8. The limit and test method are stated in the individual monograph or approved by the competent authority. Based on appropriate test validation for bacterial endotoxins and pyrogens, the test for bacterial endotoxins may replace the test for pyrogens.

Additional properties. Control of additional properties (e.g. physical characteristics, functionality-related characteristics) may be necessary for individual manufacturing processes or formulations. Grades (such as sterile, endotoxin-free, pyrogen-free) may be produced with a view to manufacture of preparations for parenteral administration or other dosage forms and appropriate requirements may be specified in an individual monograph.

Assay. Unless justified and authorised, contents of active pharmaceutical ingredients are determined using methods specified in individual monograph.

Labelling. Where appropriate, the label states that the active pharmaceutical ingredient is

— intended for a specific use;
— of a distinct crystalline form;
— of a specific degree of fineness;
— compacted;
— coated;
— granulated;
— sterile;
— free from bacterial endotoxins;
— free from pyrogens;
— containing gliding agents.

Where applicable, the label states:

— the degree of hydration;
— the name and concentration of any excipient.

Capsules

Capsules are solid dosage forms in which the drug or a mixture of drugs is enclosed in Hard Gelatin Capsule Shells, in soft, soluble shells of gelatin, or in hard or soft shells of any other suitable material, of various shapes and capacities. They usually contain a single dose of active ingredient(s) and are intended for oral administration. Capsules may also be used for other applications such as dry powder inhalers, suppositories etc. The consistency of soft shells may be adjusted by the addition of substances such as Glycerin or Sorbitol. Excipients such as opaque fillers, anti-microbial preservatives, sweetening agents, flavouring agents, processing aids and one or more colouring agents permitted under the Drugs and Cosmetic Rules, 1945 may be added. Capsules may bear surface markings.

The contents of capsules may be filled with powder, granules, pellets, beads, tablets, paste, liquid or paste-like consistency. They consist of the medicament(s) with or without excipients such as vehicles, solvents, diluents, lubricants, fillers, wetting agents and disintegrating agents. The contents should not cause deterioration of the shell, but the capsules are attacked by the digestive fluids thereby releasing the contents.

Production

During manufacture, packaging, storage and distribution of capsules, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in chapter 2.2.9.

Tests

Content of active ingredients. Determine the amount of active ingredient(s) by the method described in the Assay and calculate the amount of active ingredient(s) in each capsule. The result lies within the range for the content of active ingredient(s) stated in the monograph. This range is based on the requirement that 20 capsules, or such other number as may be indicated in the monograph, are used in the Assay. Where 20 capsules cannot be obtained, a smaller number, which must not be less than 5, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table 1. The requirements of Table 1 apply when the stated limits are between 90 and 110 per cent. For limits other than 90 to 110 per cent, proportionately smaller or larger allowances should be made.

Table 1

Weight of Active ingredients in each Capsules	Subtract from the lower limit for samples of			Add to the upper limit for samples of		
	15	10	5	15	10	5
0.12 g or less	0.2	0.7	1.5	0.3	0.8	1.8
More than 0.12 g and less than 0.3 g	0.2	0.5	1.2	0.3	0.6	1.5
0.3 g or more	0.1	0.2	0.8	0.2	0.4	1.0

Uniformity of weight (2.5.3). This test is not applicable to capsules that are required to comply with the test for Uniformity of content for all active ingredients.

Weigh an intact capsule. Open the capsule without losing any part of the shell and remove the contents as completely as possible. To remove the contents of a soft capsule the shell may be washed with *ether* or other suitable solvent and the shell allowed to stand until the odour of the solvent is no longer detectable. Weigh the shell, the weight of the contents is the difference between the weighings. Repeat the procedure with a further 19 capsules. Determine the average weight of capsule contents. Not more than two of the individual weights deviate from the average weight by more than the percentage deviation shown in Table 2 and none deviates by more than twice that percentage.

Table 2

Average weight of capsule contents	Percentage deviation
Less than 300 mg	10
300 mg or more	7.5

Uniformity of content (2.5.4). This test is applicable to capsules that contain less than 10 mg or less than 10 per cent w/w of

active ingredient. For capsules containing more than one active ingredient carry out the test for each active ingredient that corresponds to the aforementioned conditions.

The test should be carried out only after the content of active ingredient(s) in a pooled sample of the capsules has been shown to be within accepted limits of the stated content.

NOTE — The test is not applicable for capsules containing multivitamins and trace elements.

Determine the content of active ingredient in each of 10 capsules taken at random using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision. The capsules comply with the test if not more than one of the individual values thus obtained is outside the limits 85 to 115 per cent of the average value and none is outside the limits 75 to 125 per cent. If maximum of three individual values are outside the limits 85 to 115 per cent of the average value repeat the determination using another 20 capsules. The capsules comply with the test if in the total sample of 30 capsules not more than three individual values are outside the limits 85 to 115 per cent and none is outside the limits 75 to 125 per cent of the average value.

Disintegration. The disintegration test is not applicable to prolonged-release capsules. For Hard Gelatin Capsules, Soft Gelatin Capsules and Hard Cellulose Capsules for which the dissolution test (2.5.2) is included in the individual monograph, the test for Disintegration is not required.

Hard Gelatin Capsules

Hard gelatin capsules have shells consisting of two prefabricated, cylindrical sections, each of which has one rounded, closed end and one open end. Hard gelatin capsules contain the medicament(s) in the form of powders, pellets or granules, semisolids or liquids etc. Where two mutually incompatible drugs are present in the mixture, one of the drugs can be put as a tablet or pellet or in small capsule and then encapsulated with the other drug in a larger capsule.

Production

Hard gelatin capsules shells are made by a process that involves dipping shaped pins into gelatin solutions, after which the gelatin films are dried, trimmed, and removed from the pins, and the body and cap pieces are joined.

Tests

Disintegration. Comply with the disintegration test (2.5.1). Unless otherwise directed in the individual monograph use *water* as the medium. If the capsules float on the surface of the medium, a disc may be added. If the capsules adhere to the disc, attach a removable piece of stainless steel woven gauze with mesh aperture of 2.0 ± 0.2 mm to the upper plate of the basket rack assembly and carry out the test omitting the discs. Operate the apparatus for 30 minutes unless otherwise directed.

Soft Gelatin Capsules

Soft gelatin capsules made from gelatin (sometimes called softgels) or other suitable material require large-scale production methods. The soft gelatin shell is somewhat thicker than that of hard-shell capsules and may be plasticized by the addition of a polyol such as *sorbitol* or *glycerin*. The ratio of dry plasticizer to dry gelatin determines the "hardness" of the shell and may be varied to accommodate environmental conditions as well as the name of contents. Like hard shells, the shell composition may include approved dyes and pigments, opacifying agents such as *titanium dioxide*, and preservatives. Flavours may be added and up to 5 per cent sucrose may be included for its sweetness and to produce a chewable shell. Soft gelatin shells normally contain 6 per cent to 13 per cent of water.

Production

Soft gelatin capsules shells are usually formed, filled with medicament and sealed in a combined operation on machines. In some cases, shells for extemporaneous use may be prefabricated. The shells which are thicker than those of hard capsules are formed to produce capsules which are spherical, oval or cylindrical with hemispherical ends.

Soft gelatin capsules also may be manufactured in a bubble process that forms seamless spherical capsules. The shells may sometimes contain a medicament. They may contain a preservative to prevent microbial contamination.

The contents of soft capsules usually consist of liquids or solids dissolved or dispersed in suitable excipients to give a paste-like consistency. With suitable equipment, powders, granules and other dry solids also may be filled into soft-shell capsule. There may be partial migration of the constituents from the capsule contents into the shell and vice versa because of the nature of the materials and the surface in contact.

Tests

Disintegration. Comply with the disintegration test (2.5.1). Unless otherwise directed in the individual monograph use *water* as the medium. The disc may be omitted if the capsule adhere to the disc or if it is likely to be attacked by the contents of capsules. Operate the apparatus for 60 minutes unless otherwise specified in the individual monographs.

If any capsules fails to disintegrate, repeat the test on further 6 capsules. In the repeat test with additional capsules, if any

of the capsules have not disintegrated, repeat the test on a further 6 capsules, replacing *water* in the vessel with *0.1 M hydrochloric acid* or *artificial gastric juice*. The capsule pass the test if all the six have disintegrated.

Prolonged-release Capsules

Prolonged-release Capsules are hard or soft capsules in which the contents or the shell, or both, contain auxiliary substances or are prepared by a special process designed to modify the rate at which the active ingredients are released.

Tests

Dissolution (2.5.2). The test should be designed to demonstrate the appropriate release of the active substance(s). The manufacturer is expected to give specifications for drug release at 3 or more test-time points. The first point should be set after a testing period corresponding to a dissolved amount of typically 20 per cent to 30 per cent. The second point should define the dissolution pattern and should be set typically 45 per cent to 55 per cent release. The final point should ensure almost complete release that is generally understood as more than 80 per cent release.

NOTE — Above specification are non-mandatory.

Carry out the test as per the manufacturer's specification for the indicated test-times.

Gastro-resistant Capsules

Gastro-resistant Capsules are delayed-release capsules that are intended to resist the gastric artificial juice and to release their active substance or substances in the intestinal fluid. Usually they are prepared by filling capsule with granules or with particles covered with a gastro-resistant coating or in certain cases, by providing hard or soft capsules with gastro-resistant shell.

Tests

Disintegration. Comply with the disintegration test (2.5.1). Use the apparatus as described under disintegration test, using one capsule in each tube. Operate the apparatus for 2 hours without the discs in *0.1 M hydrochloric acid*. No capsule should show sign of disintegration or of rupture permitting the escape of the contents. Replace the medium in the vessel with *mixed phosphate buffer pH 6.8*. When justified and authorized, a buffer solution of pH 6.8 with added pancreas powder (for example, 0.35 g of *pancreas powder* per 100 ml of buffer solution) may be used. Add a disc to each tube and operate the apparatus for a further 60 minutes

Dissolution (2.5.2). For capsules filled with granules or particles already covered with an enteric coating, the dissolution test is carried out to demonstrate the appropriate release of the active substance(s).

Hard Cellulose Capsules

Hard Cellulose Capsule Shells are soluble containers for incorporation of drugs and food products, usually in the form of powders, pellets or granules, semisolids or liquids etc and are commonly intended for oral administration. The shells are acted upon by digestive fluids and the filled contents are released. They are composed of *Hydroxypropylmethyl-cellulose* or any other cellulose derivatives and *water*.

Hard Cellulose Capsules have shells consisting of two prefabricated, cylindrical sections, each of which has one rounded, closed end and one open end. Where two mutually incompatible drugs are present in the mixture, one of the drugs can be put as a tablet or pellet or in small capsule and then encapsulated with the other drug in a larger capsule.

Production

Hard Cellulose Capsules shells are made by a process that involves dipping shaped pins into cellulose solutions, after which the cellulose films are dried, trimmed, and removed from the pins, and the body and cap pieces are joined.

Tests

Disintegration. Comply with the disintegration test (2.5.1). Unless otherwise directed in the individual monograph use *water* as the medium. If the capsules float on the surface of the medium, a disc may be added. If the capsules adhere to the disc, attach a removable piece of stainless steel woven gauze with mesh aperture of 2 ± 0.2 mm to the upper plate of the basket rack assembly and carry out the test omitting the discs. Operate the apparatus for 30 minutes unless otherwise directed.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states (1) the name of any added antimicrobial preservative. (2) The label states the common name of the color used.

Creams

Creams are homogeneous, semi-solid or viscous preparations that possess a relatively fluid consistency and are intended for external application to the skin or certain mucous membranes for protective, therapeutic or prophylactic purposes especially where an occlusive effect is not necessary. They are semisolids usually consisting of solutions or dispersions of one or more medicaments in suitable bases*. They are formulated using hydrophilic or hydrophobic bases to provide preparations that are essentially miscible with the skin secretion.

In recent times the term cream has been restricted to products consisting of oil-in-water emulsions or aqueous microcrystalline dispersions of long-chain fatty acids or alcohols that are water-washable and more cosmetically and aesthetically acceptable. Creams can be used for administering drugs via the vaginal route.

The base should not produce irritation or sensitisation of the skin, nor should it retard wound healing; it should be smooth, inert, odourless or almost odourless, physically and chemically stable and compatible with the skin and with incorporated medicaments.

Creams may contain suitable antimicrobial preservatives unless the active ingredients or the bases themselves have sufficient bactericidal or fungicidal activity. They may contain other suitable auxiliary substances such as antioxidants, stabilisers, thickeners and emulsifiers.

If a cream is specifically intended for use on large open wounds or on severely injured skin it should be sterile.

Creams should not normally be diluted; if dilution is necessary, care should be taken to prevent instability and, in particular, microbial contamination.

Production

Creams should be packed in well-closed containers fitted with closures that minimise contamination with micro-organisms. When practicable, creams should be packed in collapsible tubes of suitable metal or plastic.

During manufacture, packaging, storage and distribution of creams, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Tests

Creams comply with the requirements of tests stated under the individual monographs and with the following requirements.

Uniformity of weight. Comply with the test for contents of packaged dosage forms (2.5.6).

Sterility. When the cream is labelled as sterile, it complies with the test for sterility (2.2.11).

Storage. Store at temperatures below 25° unless otherwise directed. Do not freeze.

Labelling. The label states (1) that the cream is sterile, where necessary; (2) the name and concentration of any added antimicrobial preservative; (3) the storage conditions.

* The term bases as a synonym for base in some of the monographs means a carrier, composed of one or more excipients, for the active pharmaceutical ingredient(s) in semi-solid and solid preparations.

Ear Drops

Otic Drops; Otic Solutions

Ear Drops are aqueous or oily solutions or suspensions of one or more medicaments intended for instillation into the outer ear. They may contain suitable auxiliary substances such as buffers, stabilising agents, dispersing agents, solubilising agents and agents to adjust the tonicity or viscosity of the preparation. However, if buffering agents are used in preparations intended for use in surgical procedures, care should be taken to ensure that the nature and concentration of the selected agents are suitable. Where the active ingredients are susceptible to oxidative degradation, a suitable antioxidant may be added but care should be taken to ensure compatibility between the antioxidant and the other ingredients of the preparations. Any additive in the preparation should not adversely affect the intended medicinal action nor, at the concentrations used, cause undue local irritation. Certain Ear Drops may be supplied in dry, sterile form to be constituted in an appropriate sterile liquid immediately before use.

Aqueous preparations supplied in multiple application containers contain suitable antimicrobial preservatives at appropriate concentrations except when the product itself has adequate antimicrobial properties. The antimicrobial preservatives should be compatible with the other ingredients of the preparation and should be effective throughout the period of use of the Ear Drops. Containers for multiple application preparations should permit the withdrawal of successive doses of the preparation. Such containers should normally hold not more than 10 ml.

During development of a formulation of ear drops containing an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated by the test for efficacy of antimicrobial preservation (2.2.2).

During manufacture, packaging, storage and distribution of ear drops, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Ear Drops intended for use in surgical procedures or for application to injured ear, are sterile. Such preparations should not contain antimicrobial preservatives and should be packed in single dose containers.

Production

Sterile Ear Drops are prepared using methods designed to ensure their sterility and to avoid the introduction of contaminants and growth of micro-organisms. Methods of sterilisation that may be used in the manufacture of Ear Drops are described in Chapter 5.3.

Description. Ear Drops that are solutions are practically clear and practically free from particles when examined under suitable conditions of visibility. Ear Drops that are suspensions may show a sediment that readily disperses when shaken. The suspension remains sufficiently dispersed to enable the correct dose to be removed from the container.

Tests

Uniformity of volume. Comply with the test for contents of packaged dosage forms (2.5.6).

Particle size. This test is applicable only to Ear Drops that are suspensions. Introduce a suitable volume of the Ear Drops into a counting cell or onto a microscope slide, as appropriate. Scan under a microscope an area corresponding to 10 µg of the solid phase. Scan at least 50 representative fields. Not more than 20 particles have a maximum dimension greater than 25 µm, not more than 10 particles have a maximum dimension greater than 50 µm and none has a maximum dimension greater than 100 µm.

Sterility. Where the label indicates that the Ear Drops are sterile, it complies with the test for sterility (2.2.11). Droppers supplied separately also comply with these tests. Remove the dropper out of the package using aseptic precautions and transfer it to a tube containing suitable culture medium so that it is completely immersed. Incubate and carry out the tests for sterility on the medium.

Storage. Ear Drops should be packed in well-closed containers. If the preparation is sterile, store in sterile, tightly-closed, tamper-evident containers. Containers should be made from materials that do not cause deterioration of the preparation as a result of diffusion into or across the material of the container or by yielding foreign substances to the preparation.

The container and package of a single application preparation should be such as to maintain sterility of the contents and the applicator up to the time of use. Containers for multiple application preparations should be fitted with an integral dropper or with a screw cap made of suitable material incorporating a dropper and plastic or rubber teat. Alternatively, such a cap assembly may be packed separately.

Labelling. The label states (1) the names and concentrations in percentages, or weight or volume per ml, of the active ingredient(s); (2) the names and concentrations of any added antioxidant, stabilising agent or antimicrobial preservative; (3) that, for multiple application containers, the contents should not be used for more than 1 month after opening the container; (4) that, for multiple application containers, care should be taken to avoid contamination of the contents during use; (5) that the preparation is NOT FOR INJECTION; (6) that, where applicable, the preparation is sterile; (7) the storage conditions.

Eye Drops

Ophthalmic Drops

Eye Drops are sterile, aqueous or oily solutions or suspensions of one or more medicaments intended for instillation into the conjunctival sac. They may contain suitable auxiliary substances such as buffers, stabilising agents, solubilising agents and agents to adjust the tonicity or viscosity of the preparation. However, if buffering agents are used in preparations intended for use in surgical procedures care should be taken to ensure that the nature and concentration of the selected agents are suitable. Where the active ingredient is susceptible to oxidative degradation, a suitable antioxidant may be added but care should be taken to ensure compatibility between the antioxidant and the other ingredients of the preparation. Any additive in the preparation should not adversely affect the intended medicinal action nor, at the concentrations used, cause undue local irritation. Certain Eye Drops may be supplied in dry, sterile form to be constituted in an appropriate sterile liquid immediately before use.

Aqueous preparations supplied in multiple application containers contain suitable antimicrobial preservatives at appropriate concentrations except when the product itself has adequate antimicrobial properties. The antimicrobial preservatives should be compatible with the other ingredients of the preparation and should be effective throughout the period of use of the Eye Drops.

If the preparation does not contain an antimicrobial preservative it should be packed in single application containers. Eye Drops intended for use in surgical procedures should not contain antimicrobial preservatives and should be packed in single application containers.

Eye Drops are prepared using methods designed to ensure their sterility and to avoid the introduction of contaminants and growth of micro-organisms. Methods of sterilisation that may be used in the manufacture of Eye Drops are described in Chapter 5.3.

Containers. Eye Drops should be packed in tamper-evident containers. Containers should be made from materials that do not cause deterioration of the preparation as a result of diffusion into or across the material of the container or by yielding foreign substances to the preparation.

The container and package of a single dose preparation should be such as to maintain sterility of the contents and the applicator up to the time of use. Containers for multiple application preparations should be fitted with an integral dropper or with a sterile screw cap of suitable materials incorporating a dropper and plastic or rubber teat. Alternatively, such a cap assembly may be packed separately after it is sterilised. Containers of multiple application preparations should permit the withdrawal of successive doses

of the preparation. Such containers should normally hold not more than 10 ml.

Description. Eye Drops that are solutions are practically clear and practically free from particles when examined under suitable conditions of visibility. Eye Drops that are suspensions may show a sediment that readily disperses when shaken. The suspension remains sufficiently dispersed to enable the correct dose to be removed from the container.

Tests

Uniformity of volume. Comply with the test for contents of packaged dosage forms (2.5.6).

Particle size. This test is applicable only to Eye Drops that are suspensions. Introduce a suitable volume of the Eye Drops into a counting cell or onto a microscope slide, as appropriate. Scan under a microscope an area corresponding to 10 µg of the solid phase. Scan at least 50 representative fields. Not more than 20 particles have a maximum dimension greater than 25 µm, not more than 10 particles have a maximum dimension greater than 50 µm and none has a maximum dimension greater than 100 µm.

Sterility. Comply with the test for sterility (2.2.11). Droppers supplied separately also comply with these tests. Remove the dropper out of the package using aseptic precautions and transfer it to a tube containing suitable culture medium so that it is completely immersed. Incubate and carry out the test.

Storage. Store in sterile containers sealed so as to protect from micro-organisms.

Labelling. The label states (1) the names and concentrations in percentages, or weight or volume per ml, of the active ingredients; (2) the names and concentrations of any added antimicrobial preservative; (3) that, for multiple application containers, the contents should not be used for more than 1 month after opening the container; (4) that, for multiple application containers, care should be taken to avoid contamination of the contents during use; (5) that the preparation is NOT FOR INJECTION; (6) the conditions under which the preparation should be stored.

Eye Ointments

Ophthalmic Ointments

Eye Ointments are sterile, semi-solid preparations of homogenous appearance intended for application to the eye. They may contain one or more medicaments dissolved or dispersed in a suitable basis. Bases, which are usually non-aqueous, may contain suitable auxiliary substances such as stabilising agents, antimicrobial preservatives and antioxidants. The base selected must be non-irritant to the conjunctiva, allow the drug to diffuse throughout the secretions of the eye and retain the activity of the medicaments for a reasonable period of time under the stated conditions of storage.

Eye Ointments are prepared using methods designed to ensure their sterility and to avoid the introduction of contaminants and growth of micro-organisms. Methods of sterilisation that may be used in the manufacture of Eye Ointments are described in Chapter 5.3.

Containers. Eye Ointments should be packed in small, sterilised collapsible tubes of metal or of suitable plastic fitted or provided with a nozzle of suitable shape to facilitate the application of the product without contamination and with a cap. The content of such containers is not more than 5 g of the preparation. Eye Ointments may also be packed in single application containers of such a shape as to facilitate administration without contamination; such containers may be individually wrapped. Other requirements concerning containers are given in Chapter 6.2.

Tests

Uniformity of weight. Comply with the test for contents of packaged dosage forms (2.5.6).

Particle size. Gently spread a small quantity of the Eye Ointment as a thin layer on a microscope slide. Scan under a microscope an area corresponding to 10 µg of the solid phase. Scan at least 50 representative fields. Not more that 20 particles have a maximum dimension greater than 25 µm, not more than 10 particles have a maximum dimension greater than 50 µm and none has a maximum dimension greater than 100 µm.

Sterility (2.2.11). Comply with the test for sterility.

Storage. Store at temperatures below 30° unless otherwise directed. Do not freeze.

Gels

Gels are homogeneous, semi-solid preparations usually consisting of solutions or dispersions of one or more medicaments in suitable hydrophilic or hydrophobic bases. They are normally prepared with the aid of suitable gelling agents. They are intended to be applied to the skin or certain mucous membranes for protective, prophylactic or therapeutic purposes. Gels may contain suitable added substances such as antioxidants, stabilisers and antimicrobial preservatives.

During manufacture, packaging, storage and distribution of gels, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Gels specifically intended for use on large open wounds or on severely injured skin should be sterile.

Containers. Gels should be packed in suitable well-closed or, if the preparation contains water or other volatile ingredients, suitable tightly-closed containers. The containers should be fitted with closures that minimise contamination with micro-organisms. To the extent possible, collapsible tubes of suitable metal or plastic should be used.

Storage. Store at temperatures below 30° unless otherwise directed. Do not freeze.

Labelling. The label states (1) that the gel is sterile, where necessary; (2) the storage conditions.

Tests

Uniformity of weight. Comply with the test for contents of packaged dosage forms (2.5.6).

Sterility. Gels labelled as sterile comply with the test for sterility (2.2.11).

Granules

Requirements for granules to be used for the preparation of oral solutions or suspensions are given in the general monograph on Oral Liquids.

Definition

Granules are preparations consisting of solid, dry aggregates of powder particles sufficiently resistant to withstand handling. They are intended for oral administration. Some are swallowed as such, some are chewed and some are dissolved or dispersed in water or another suitable liquid before being administered.

Granules contain one or more active substances with or without excipients and, if necessary, colouring matter authorized by the competent authority and flavouring substances.

Granules are presented as single-dose or multi-dose preparations. Each dose from a multidose preparation is administered by means of a device suitable for measuring the quantity prescribed. For single-dose granules, each dose is enclosed in an individual container, for example a sachet or a vial.

Where applicable, containers for granules comply with the requirements of Containers for Pharmaceutical Products (chapter 6.2)

Several categories of granules may be distinguished:

— effervescent granules;
— coated granules;
— modified-release granules.
— gastro-resistant granules;
— immediate-release granules.

Production

In the manufacture, packaging, storage and distribution of granules, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Tests

Uniformity of content (2.5.4) Unless otherwise prescribed or justified and authorised, single-dose granules with a content of active substance less than 10 mg or less than 10 per cent of the total mass comply with test for uniformity of content of single-dose preparations. For granules containing more than one active ingredient, carry out the test for each active ingredient that corresponds to the aforementioned conditions.

Uniformity of container contents. Granules supplied in multidose containers comply with the test for contents of packaged dosage forms (2.5.6).

Effervescent Granules

Effervescent granules are uncoated granules generally containing acid substances and carbonates or hydrogen carbonates which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

Tests

Disintegration (2.5.1) Place one dose of the effervescent granules in a beaker containing 200 ml of *water* at 15-25°; numerous bubbles of gas are evolved. When the evolution of gas around the individual grains ceases, the granules have disintegrated, being either dissolved or dispersed in the *water.* Repeat the operation on 5 other doses. The preparation complies with the test if each of the 6 doses used disintegrates within 5 minutes.

Coated Granules

Coated granules are usually multidose preparations and consist of granules coated with one or more layers of mixtures of various excipients.

Production

The substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs.

Tests

Dissolution (2.5.2). Where required, the requirements for this test are given in the individual monograph.

Modified-release Granules

Modified-release granules are coated or uncoated granules which contain special excipients or which are prepared by special procedures, or both, designed to modify the rate, the place or the time at which the active substance or substances are released.

Modified-release granules include prolonged-release granules and delayed-release granules.

Production

A suitable test is carried out to demonstrate the appropriate release of the active substance(s).

Tests

Dissolution (2.5.2). Where required, the requirements for this test are given in the individual monograph.

Gastro-resistant Granules

Gastro-resistant granules are delayed-release granules that are intended to resist the gastric artificial juice and to release the active substance(s) in the intestinal fluid. These properties are achieved by covering the granules with a gastro-resistant material (enteric-coated granules) or by other suitable means.

Production

A suitable test is carried out to demonstrate the appropriate release of the active substance(s).

Tests

Dissolution (2.5.2). Where required, the requirements for this test are given in the individual monograph.

Immediate-release Granules

Definition

Granules are preparations consisting of solid, dry aggregates of powder particles sufficiently resistant to withstand handling. They are intended for oral administration. Some are swallowed as such; some are dissolved or dispersed in water or another suitable liquid before being administered.

Granules contains one or more active substances with or without excipients and, if necessary, coloring matter authorized by the competent authority and flavouring substances.

Granules are presented as single-dose or multi-dose preparations. Each dose of a multi-dose preparation is administered by means of a device suitable for measuring the quantity prescribed. For single-dose granules, each dose is enclosed in an individual container, for example a sachet or a vial.

Where applicable, containers for granules comply with the requirements of Materials used for the manufacture of containers and Containers.

Several categories of granules may be distinguished:

— Effervescent granules;

— *Coated granules.*

Production

In the manufacture, packaging, storage and distribution of granules, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in the text on microbial contamination in nonsterile products (2.2.9).

Tests

Uniformity of content (2.5.4). Unless otherwise prescribed or justified and authorized, single-dose granules with a content of active substance less than 10 mg or less than 10 per cent of the total mass comply with test for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances, which correspond to the above conditions.

Uniformity of weight (2.5.3). Single-dose granules except for coated granules comply with the test for uniformity of weight of single-dose preparations. If the test for uniformity of content is prescribed for all active substances, the test for uniformity of weight is not required.

Uniformity of container contents. Granules supplied in multidose containers comply with the test for contents of packaged dosage forms (2.5.6).

Storage. All types of granules should be stored in airtight container to prevent unusual and other changes before packing, unless otherwise stated in the individual monograph.

For Immediate Release Granules, if the preparation contains volatile ingredients or the contents have to be protected, store in an airtight container.

Inhalation Preparations

Inhalation Preparations are liquid or solid preparations intended for administration as vapours or aerosols to the lung in order to obtain a local or systemic effect. They contain one or more active substances that may be dissolved or dispersed in a suitable vehicle.

Inhalation Preparations may, depending on the type of preparation, contain propellants, cosolvents, diluents,

antimicrobial preservatives, solubilising and stabilising agents, etc. These excipients do not adversely affect the functions of the mucosa of the respiratory tract or its cilia. Suspensions and emulsions are readily dispersible on shaking and they remain sufficiently stable to enable the correct dose to be delivered. Inhalation Preparations are supplied in single-dose or multidose containers.

Inhalation Preparations intended to be administered as aerosols (dispersions of solid or liquid particles of active ingredient(s) in a gas) are administered by one of the following devices: a nebuliser; an inhaler (pressurised metered-dose inhaler, non-pressurised metered-dose inhaler or powder inhaler).

Several categories of Inhalation Preparations may be distinguished: preparations to be converted into vapour; liquid preparations for nebulisation; pressurised metered-dose preparations for inhalation; non-pressurised metered-dose preparations for inhalation; inhalation powders.

Production

Inhalation preparations should be manufactured in conditions designed to minimise microbial and particulate contamination.

During the development of a preparation that contains an antimicrobial preservative, the effectiveness of the preservative selected, shall be determined as described in chapter 2.2.2. Effectiveness of antimicrobial preservatives.

In the manufacture, packaging, storage and distribution of preparations for inhalation, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in chapter 2.2.9. Microbial contamination in Nonsterile products.

Uniformity of delivered dose of a multidose inhaler must be ensured within a device (intra-inhaler) and between devices (inter-inhaler). For intra-inhaler testing, the uniformity of delivered dose tests are described in the Tests sections of the various preparation categories in this monograph. For inter-inhaler testing, a suitable procedure is to take 10 inhalers and collect a single dose from each inhaler, collecting the dose at the beginning (from 3 inhalers), middle (from 4 inhalers) and end (from 3 inhalers) of the number of doses stated on the label. Other inter-inhaler testing procedures are possible, where justified.

Storage. Avoid storage under extremes of temperature and in an environment with undue fluctuations in temperature.

Labelling. The label states (1) the name(s) of the active ingredient(s); (2) the total amount of the active ingredient(s) in the container except in the case of metered-dose preparation for inhalation); (3) that the container should be shaken before use; (4) the other instructions for use; (5) the date after which the preparation is not intended to be used; (6) the conditions under which it should be stored; (7) a warning that the container is under pressure and that it must not be punctured, broken or incinerated even when apparently empty; (8) the statement "Warning. Keep away from children".

In the case of metered-dose aerosols and pressurized metered dose inhalers, the label states in addition (1) the total number of deliveries available from the container; (2) the amount of active ingredient(s) released each time the valve is actuated.

In the case of dry powder inhalers the label on the container states (1) the date after which the dry powder inhaler is not intended to be used; (2) the conditions under which the powder for Inhalation should be stored. Where the powder for Inhalation is supplied in a capsule, the label also states; (3) the quantity of the active ingredient contained in each capsule; (4) that the capsules are intended for use in an inhaler and are not to be swallowed.

Information on use of the preparation provided in the pack shall include (1) the direction for correct use of the aerosol; (2) a warning that the container may explode if punctured, exposed to excessive heat or direct sunlight; (3) the directions for the disposal of the used or partly-used container.

Preparations to be converted into vapour

Preparations intended to be converted into vapour are liquids, solutions, suspensions, emulsions, or semi-solid or solid preparations. They are usually added to hot water and the vapour generated is inhaled.

Liquid preparations for nebulisation

Liquid preparations for nebulisation are solutions, suspensions or emulsions intended to be converted into aerosols by nebulisers.

Liquid preparations for nebulisation in concentrated form are diluted to the prescribed volume with the prescribed liquid before use. Liquid preparations for nebulisation may also be prepared from powders by reconstitution in the prescribed liquid.

The pH of liquid preparations for nebulisation is not lower than 3 and not higher than 10.

Liquid preparations for nebulisation supplied in multi-dose containers may contain a suitable antimicrobial preservative at a suitable concentration except where the preparation itself has adequate antimicrobial properties.

Liquid preparations for nebulisation supplied in multi-dose containers that do not contain an antimicrobial preservative, and where the preparation itself does not have adequate antimicrobial properties, are sterile and are supplied in containers preventing microbial contamination of the contents during storage and use.

Liquid preparations for nebulisation supplied in single-dose containers are sterile and preservative-free, unless otherwise justified and authorised.

Nebulisers are devices that convert liquids into aerosols by high-pressure gases, ultrasonic vibration, and extrusion through a mesh or other methods. They allow the dose to be inhaled at an appropriate active-substance delivery rate over an extended period of time involving consecutive inhalations and with a particle size that allows deposition of the preparation in the lungs.

Nebulisers may be breath-triggered or use other means to synchronise or modify the nebuliser operation with the patient's breathing.

Production

The active substance delivery rate, the total active substance delivered and the particle per droplet-size distribution are determined using the methods described in Preparations for nebulisation: characterisation. Where justified and authorised, different apparatus and procedures may be used.

Tests

Prepare the liquid preparation for nebulisation as directed in the instructions to the patient.

Uniformity of content (2.5.4). The test is applicable to Nebulisers that contain less than 10 mg or less than 10 per cent of active ingredient. For Nebulisers containing more than one active ingredient, carry out the test for each active ingredient that corresponds to the above conditions.

The test for uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the nebulisers has been shown to be within accepted limits of stated content.

Uniformity of weight (2.5.3). This test is not applicable to Nebulisers that are required to comply with the test for Uniformity of content for all the active ingredients.

Weigh individually the contents of 20 containers, emptied as completely as possible, and determine the average weight; not more than two of the individual weights deviate from the average weight by more than 10 per cent and none deviate by more than 20 per cent.

Aerodynamic assessment of nebulised aerosols

For liquid preparations for nebulisation that are suspensions, determine fine-particles mass using an apparatus and procedure described in preparations for nebulisation: characterization. Where justified and authorised, a different apparatus and procedure may be used.

Pressurised metered-dose preparations for inhalation

Pressurised metered-dose preparations for inhalation are solutions, suspensions or emulsions supplied in containers equipped with a metering valve and which are held under pressure with (a) suitable propellant(s), which can act also as a solvent.

The delivered dose is the dose delivered from the inhaler. For some preparations the dose has been established as a metered dose. The metered dose is determined by adding the amount deposited on the inhaler to the delivered dose. It may also be determined directly.

Production

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lungs. The fine-particle characteristics of pressurised metered-dose preparations for inhalation are determined using the method described in Preparations for inhalation: aerodynamic assessment of fine particles.

Tests

For breath-triggered pressurised metered-dose inhalers, the test conditions described below may need to be modified to ensure that actuation occurs for the inhaler under test.

Prepare the inhaler as directed in the instructions to the patient.

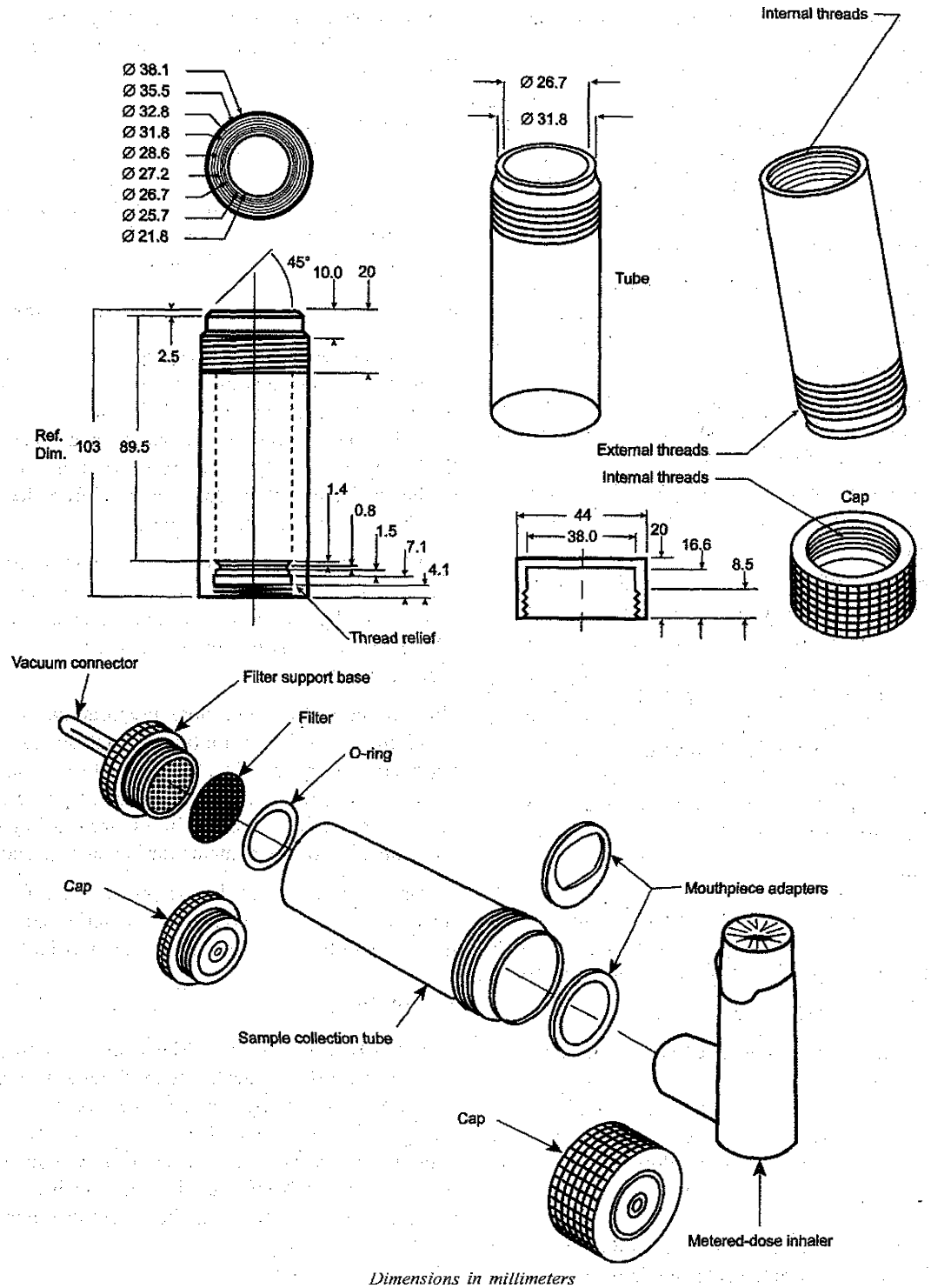
Uniformity of delivered dose

Pressurised metered-dose inhalers usually operate in a valve-down position. For inhalers that operate in a valve-up position, an equivalent test is applied using methods that ensure the complete collection of the delivered dose.

The dose collection apparatus must be capable of quantitatively capturing the delivered dose.

The following apparatus (Fig. 1) and procedure may be used.

The apparatus consists of a filter-support base with an open-mesh filter-support, such as a stainless steel screen, a collection tube that is clamped or screwed to the filter-support base, and a mouthpiece adapter to ensure an airtight seal between the collection tube and the mouthpiece. Use a mouthpiece adapter that ensures that the front face of the inhaler mouthpiece is flush with the front face or the 2.5 mm indented shoulder of the sample collection tube, as appropriate. The vacuum connector is connected to a system comprising a vacuum source and a flow regulator. The source is adjusted to draw air through the complete assembly, including the filter and the inhaler to be tested, at 28.3 litres per minute (± 5 per cent). Air should be drawn continuously through the apparatus to avoid loss of the active substance into the atmosphere. The filter-support base is designed to accommodate 25 mm diameter filter disks. The filter disk and other materials used in the



Dimensions in millimeters

Fig. 1: Dose collection apparatus for pressurized metered-dose inhalers

construction of the apparatus must be compatible with the active substance and solvents that are used to extract the active substance from the filter. One end of the collection tube is designed to hold the filter disk tightly against the filter-support base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection tube passes through the inhaler.

Unless otherwise prescribed in the instructions to the patient, shake the inhaler for 5 seconds and discharge 1 delivery to waste. Discharge the inverted inhaler into the apparatus, depressing the valve for a sufficient time to ensure complete discharge. Repeat the procedure until the numbers of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste, waiting not less than 5 seconds between actuations, until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste, waiting not less than 5 seconds between actuations, until 3 doses remain. Collect these 3 doses using the procedure described above.

For preparations containing more than 1 active substance, carry out the test for uniformity of delivered dose for each active substance.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the average value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent. Unless otherwise authorised, the mean value must be between 85 per cent and 115 per cent of the label claim for delivered dose.

Fine particle dose

Using an apparatus and procedure described in *Preparations for inhalation: aerodynamic assessment of fine particles* (apparatus B, C or D), calculate the fine particle dose.

Number of deliveries per inhaler

Take 1 inhaler and discharge the contents to waste, actuating the valve at intervals of not less than 5 seconds. The total number of deliveries so discharged from the inhaler is not less than the number stated on the label (this test may be combined with the test for uniformity of delivered dose).

Leak rate

Take a suitable number of containers, for example 1 container, remove any labels and record the date and time to the nearest half hour. Weigh the container to the nearest milligram and record the mass (M_1) in milligrams. Allow the containers to stand in an upright position at a temperature of 25.0 ± 2.0° for not less than 3 days, and again weigh the container, recording the mass (M_2) in milligrams, and recording the date and time to the nearest half hour. Determine the time (T), in hours, during which the container was under test.

Calculate the total loss of mass, in milligrams, over the entire shelf life (D), in months, of the container, using the following expression:

$$\frac{730 \times D}{T} \times (M_1 - M_2)$$

Unless otherwise justified, the preparation complies if the total loss of mass over the entire shelf life is not more than 10 per cent (m/m) of the nominal fill mass of the container.

Non-pressurised metered-dose preparations for inhalation

Non-pressurised metered-dose preparations for inhalation are solutions, suspensions or emulsions for use with inhalers that convert liquids into aerosols using single or multiple liquid jets, ultrasonic vibration or other methods. The volume of liquid to be converted into an aerosol is pre-metered or metered by the inhaler so that the dose delivered from the inhaler can be inhaled with 1 or more inspirations.

Non-pressurised metered-dose preparations for inhalation supplied in multidose containers may contain a suitable antimicrobial preservative at a suitable concentration except where the preparation itself has adequate antimicrobial properties.

Non-pressurised metered-dose preparations for inhalation supplied in multidose containers that do not contain an antimicrobial preservative and where the preparation itself does not have adequate antimicrobial properties, are sterile and are supplied in containers preventing microbial contamination of the contents during storage and use.

Non-pressurised metered-dose preparations for inhalation supplied in single-dose containers are sterile and preservative-free, unless otherwise justified and authorised.

Production

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lung. The fine-particle characteristics of non-pressurised metered-dose preparations for inhalation are determined using the method described in *Preparations for inhalation: aerodynamic assessment of fine particles*. Alternatively, laser diffraction analysis may be used, when properly validated against method (apparatus B, C or D).

Tests

For breath-triggered non-pressurised metered-dose inhalers, the test conditions described below may need to be modified to ensure that actuation occurs for the inhaler under test.

Prepare the inhaler as directed in the instructions to the patient.

Uniformity of delivered dose

The dose collection apparatus must be capable of quantitatively capturing the delivered dose. The apparatus described in the test for uniformity of delivered dose for pressurised metered-dose preparations may be used.

Discharge the inhaler into the apparatus. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste until 3 doses remain. Collect these 3 doses using the procedure described above.

For preparations containing more than 1 active substance, carry out the test for uniformity of delivered dose for each active substance.

Unless otherwise justified and authorised, the preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the average value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent.

Where justified and authorised, another apparatus and procedure may be used.

Fine particle dose

Using an apparatus and procedure described in *Preparations for inhalation: aerodynamic assessment of fine particles* (apparatus B, C or D), calculate the fine particle dose. Use the same procedure as for pressurised inhalers with appropriate adaptation of the methodology to non-pressurised inhalers. Depending on the characteristics of the non-pressurised metered-dose preparations for inhalation, relative humidity and/or temperature may need to be controlled during the test.

Number of deliveries per inhaler

Take 1 inhaler and discharge the contents to waste. The total number of deliveries so discharged from the inhaler is not less than the number stated on the label (this test may be combined with the test for uniformity of delivered dose).

Inhalation powders

Inhalation powders are supplied in single-dose or multidose containers. To facilitate their use, active substances may be combined with a suitable carrier. They are administered by powder inhalers. For pre-metered inhalers, the inhaler is loaded with powders pre-dispensed in capsules or other suitable dosage forms. For inhalers using a powder reservoir, the dose is created by a metering mechanism within the inhaler.

The delivered dose is the dose delivered from the inhaler. For some preparations, the labelled dose has been established as a metered dose or as a pre-dispensed dose. The metered dose is determined by adding the amount deposited on the inhaler to the delivered dose. It may also be determined directly.

Production

The size of aerosol particles to be inhaled is controlled so that a consistent portion is deposited in the lung. The fine-particle characteristics of powders for inhalation are determined using the method described in general chapter *Preparations for inhalation: aerodynamic assessment of fine particles.*

Tests

Prepare the inhaler as directed in the instructions to the patient.

Uniformity of delivered dose

The dose collection apparatus must be capable of quantitatively capturing the delivered dose. A dose collection apparatus similar to that described for the evaluation of pressurised metered-dose inhalers may be used provided that the dimensions of the tube and the filter can accommodate the measured flow rate. A suitable tube is defined in Table 1. Connect the tube to a flow system according to the scheme specified in Fig. 2 and Table 1.

Unless otherwise stated, determine the test flow rate and duration using the dose collection tube, the associated flow system, a suitable differential pressure meter and a suitable volumetric flow meter, calibrated for the flow leaving the meter, according to the following procedure.

Prepare the inhaler for use and connect it to the inlet of the apparatus using a mouthpiece adapter to ensure an airtight seal. Use a mouthpiece adapter that ensures that the front face of the inhaler mouthpiece is flush with the front face of the sample collection tube. Connect one port of a differential pressure meter to the pressure reading point P1 in Fig. 2, and let the other be open to the atmosphere. Switch on the pump, open the 2-way solenoid valve and adjust the flow control valve until the pressure drop across the inhaler is 4.0 kPa (40.8 cm H_2O) as indicated by the differential pressure meter. Remove the inhaler from the mouthpiece adapter and, without touching the flow control valve, connect a flowmeter to the inlet of the sampling apparatus. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric

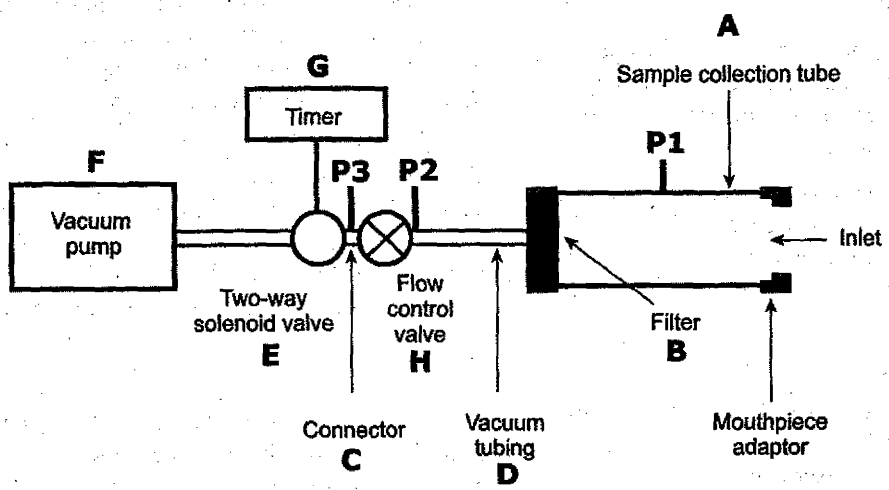


Fig. 2: Apparatus for measuring the uniformity of delivered dose for powders for inhalation

Table 1 – Specifications of the apparatus used for powder for inhalation described in Fig. 2

Code	Item	Description
A	Sample collection tube	Capable of quantitatively capturing the delivered dose, e.g. dose collection tube similar to that described in Figure A with dimensions of 34.85 mm ID x 12 cm length (e.g. product number XX40 047 00, Millipore Corporation, Bedford, MA 01732 with modified exit tube, ID ≥ 8 mm, fitted with Gelman product number 61631), or equivalent.
B	Filter	47 mm filter, e.g. A/E glass fibre filter (Gelman Sciences, Ann Arbor, MI 48106), or equivalent.
C	Connector	ID ≥ 8 mm, e.g. short metal coupling, with low-diameter branch to P3
D	Vacuum tubing	A length of suitable tubing having an ID ≥ 8 mm and an internal volume of 25 ± 5 ml
E	2-way solenoid valve	A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with ID ≥ 8 mm and an opening time ≤ 100 ms (e.g. type 256-A08, Burkert GmbH, D-74653 Ingelfingen), or equivalent.
F	Vacuum pump	Pump must be capable of drawing the required flow rate through the assembled apparatus with the powder inhaler in the mouthpiece adapter (e.g. product type 1023, 1423 or 2565, GAST Manufacturing Inc., Benton Harbor, MI 49022), or equivalent. Connect the pump to the 2-way solenoid valve using short and/or wide (≥ 10 mm ID) vacuum tubing and connectors to minimize pump capacity requirements.
G	Timer	Timer capable of driving the 2-way solenoid valve for the required time period (e.g. type G814, RS Components International, Corby, NN17 9 RS, UK), or equivalent.
P1	Pressure tap	2.2 mm ID, 3.1 mm OD, flush with internal surface of the sample collection tube, centred and burr-free, 59 mm from its inlet. The pressure tap P1 must never be open to the atmosphere.
P2, P3	Pressure measurements	Differential pressure to atmosphere (P1) or absolute pressure (P2 and P3)
H	Flow control valve	Adjustable regulating valve with maximum Cv ≥1, (e.g. type 8FV12LNSS, Parker Hannifin plc., Barnstaple, EX31 1NP, UK), or equivalent.

flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

Where, P_0 = Atmospheric pressure;
ΔP = Pressure drop over the meter.

If the flow rate is above 100 liters per minutes adjust the flow control valve to obtain a flow rate of 100 liter per minutes (± 5 per cent). Note the volumetric airflow rate exiting the meter and define this as the test flow rate, Q_{out}, in litres per minute. Define the test flow duration, T, in seconds so that a volume of 4 litre of air is drawn from the mouthpiece of the inhaler at the test flow rate, Q_{out}.

Ensure that critical flow occurs in the flow control valve by the following procedure: with the inhaler in place and the test flow rate Q_{out}, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Fig. 2); a ratio P3/P2 of less than or equal to 0.5 indicates critical flow; switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

Pre-metered inhalers. Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler using the predetermined conditions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance.

Repeat the procedure for a further 9 doses.

Device-metered inhalers. Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance. Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. If necessary, store the inhaler to discharge electrostatic charges. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste until 3 doses remain. If necessary, store the inhaler to discharge electrostatic charges. Collect 3 doses using the procedure described above.

For preparations containing more than 1 active substance, carry out the test for uniformity of delivered dose for each active substance.

Results. The preparation complies with the test if 9 out of 10 results lie between 75 per cent and 125 per cent of the average value and all lie between 65 per cent and 135 per cent. If 2 or 3 values lie outside the limits of 75 per cent to 125 per cent, repeat the test for 2 more inhalers. Not more than 3 of the 30 values lie outside the limits of 75 per cent to 125 per cent and no value lies outside the limits of 65 per cent to 135 per cent.

In justified and authorised cases, these ranges may be extended but no value should be greater than 150 per cent or less than 50 per cent of the mean value. Unless otherwise authorized, the mean value must be between 85 per cent and 115 per cent of the label claim for delivered dose.

Fine particle dose

Using an apparatus and procedure described in *Preparations for inhalation: aerodynamic assessment of fine particles* (apparatus B, C or D), calculate the fine particle dose.

Number of deliveries per inhaler for multidose inhalers

Discharge doses from the inhaler until empty, at the predetermined flow rate. Record the deliveries discharged. The total number of deliveries so discharged from the inhaler is not less than the number stated on the label (this test may be combined with the test for uniformity of delivered dose).

Preparations for Inhalation: Aerodynamic Assessment of Fine Particles

This test is used to determine the fine particle characteristics of the aerosol clouds generated by preparations for inhalation.

Unless otherwise justified and authorised, one of the following apparatus and test procedures is used.

Stage mensuration. Is performed periodically together with confirmation of other dimensions critical to the effective operation of the impactor.

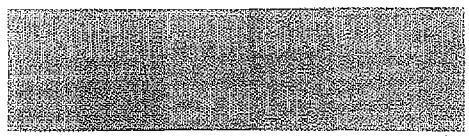
Re-entrainment (for apparatus B and D). To ensure efficient particle capture, coat each plate with glycerol, silicone oil or similar high viscosity liquid, typically deposited from a volatile solvent. Plate coating must be part of method validation and may be omitted where justified and authorised.

Mass balance. The total mass of the active substance is not less than 75 per cent and not more than 125 per cent of the average delivered dose determined during testing for uniformity of delivered dose. This is not a test of the inhaler but it serves to ensure that the results are valid.

Apparatus A. Glass Impinger

Procedure for Nebulisers

Introduce 7 ml and 30 ml of a suitable solvent into the upper and lower impingement chambers, respectively.



Connect all the component parts. Ensure that the assembly is vertical and adequately supported and that the jet spacer peg of the lower jet assembly just touches the bottom of the lower impingement chamber. Connect a suitable pump fitted with a filter (of suitable pore size) to the outlet of the apparatus. Adjust the air flow through the apparatus, as measured at the inlet to the throat, to 60 ± 5 litres per minute.

Introduce the liquid preparation for inhalation into the reservoir of the nebuliser. Fit the mouthpiece and connect it by means of an adapter to the device.

Switch on the pump of the apparatus and after 10 seconds switch on the nebuliser.

After 60 seconds, unless otherwise justified, switch off the nebuliser, wait for about 5 seconds and then switch off the pump of the apparatus. Dismantle the apparatus and wash the inner surface of the upper impingement chamber collecting the washings in a volumetric flask. Wash the inner surface of the lower impingement chamber collecting the washings in a second volumetric flask. Finally, wash the filter preceding the pump and its connections to the lower impingement chamber and combine the washings with those obtained from the lower impingement chamber. Determine the amount of active substance collected in each of the 2 flasks. Express the results for each of the 2 parts of the apparatus as a percentage of the total amount of active substance.

Procedure for pressurised inhalers

Place the actuator adapter in position at the end of the throat so that the mouthpiece end of the actuator, when inserted to a depth of about 10 mm, lines up along the horizontal axis of the throat and the open end of the actuator, which accepts the pressurised container, is uppermost and in the same vertical plane as the rest of the apparatus.

Introduce 7 ml and 30 ml of a suitable solvent into the upper and lower impingement chambers, respectively.

Connect all the component parts. Ensure that the assembly is vertical and adequately supported and that the lower jet-spacer peg of the lower jet assembly just touches the bottom of the lower impingement chamber. Connect a suitable pump to the outlet of the apparatus. Adjust the air flow through the apparatus, as measured at the inlet to the throat, to 60 ± 5 litres per minute.

Prime the metering valve by shaking for 5 seconds and discharging once to waste; after not less than 5 seconds, shake and discharge again to waste. Repeat a further 3 times.

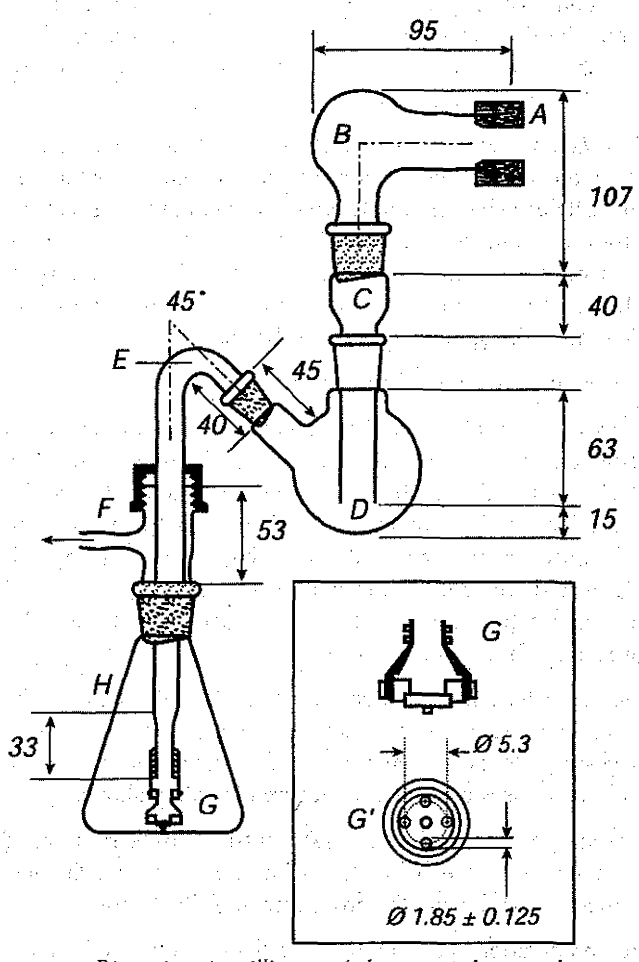
Shake for about 5 seconds, switch on the pump to the apparatus and locate the mouthpiece end of the actuator in the adapter, discharge once immediately. Remove the assembled inhaler from the adapter, shake for not less than 5 seconds, relocate the mouthpiece end of the actuator in the adapter and discharge again. Repeat the discharge sequence. The number of discharges should be minimised and typically would not be greater than 10. After the final discharge wait for not less than 5 seconds and then switch off the pump. Dismantle the apparatus.

Wash the inner surface of the inlet tube to the lower impingement chamber and its outer surface that projects into the chamber with a suitable solvent, collecting the washings in the lower impingement chamber. Determine the content of active substance in this solution. Calculate the amount of active substance collected in the lower impingement chamber per discharge and express the results as a percentage of the dose stated on the label.

Procedure for Powder Inhalers

Introduce 7 ml and 30 ml of a suitable solvent into the upper and lower impingement chambers, respectively.

Connect all the component parts. Ensure that the assembly is vertical and adequately supported and that the jet-spacer peg of the lower jet assembly just touches the bottom of the lower



Dimensions in millimetres (tolerances ± 1 mm, unless otherwise prescribed)

Fig. 3: Apparatus A. Glass impinger

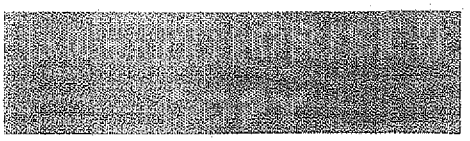
impingement chamber. Without the inhaler in place, connect a suitable pump to the outlet of the apparatus. Adjust the air flow through the apparatus, as measured at the inlet to the throat, to 60 ± 5 litres per minute.

Prepare the inhaler for use and locate the mouthpiece in the apparatus by means of a suitable adapter. Switch on the pump for 5 seconds. Switch off the pump and remove the inhaler. Repeat the discharge sequence. The number of discharges

Table 2 – Component specification for apparatus A in Fig. 3

Code	Item	Description	Dimensions*
A	Mouthpiece adaptor	Moulded rubber adapter for actuator mouthpiece.	
B	Throat	Modified round-bottomed flask:	50 ml
		ground-glass inlet socket	29/32
		ground-glass outlet cone	24/29
C	Neck	Modified glass adapter:	
		ground-glass inlet socket	24/29
		ground-glass outlet cone	24/29
		Lower outlet section of precision-bore glass tubing:	
		bore diameter	14
		Selected bore light-wall glass tubing:	
		external diameter	17
D	Upper impingement chamber	Modified round-bottomed flask	100 ml
		ground-glass inlet socket	24/29
		ground-glass outlet cone	24/29
E	Coupling tube	Medium-wall glass tubing:	
		ground-glass cone	14/23
		Bent section and upper vertical section:	
		external diameter	13
		Lower vertical section:	
		external diameter	8
F	Screw thread, side-arm adaptor	Plastic screw cap	28/13
		Silicone rubber ring	28/11
		PTFE washer	28/11
		Glass screw thread:	
		thread size	28
		Side-arm outlet to vacuum pump:	
		minimum bore diameter	5
G	Lower jet assembly	Modified polypropylene filter holder connected to lower vertical section of coupling tube by PTFE tubing	See Fig. 3
		Acetal circular disc with the centres of four jets arranged on a projected circle of diameter 5.3 mm with an integral jet spacer peg:	10
		peg diameter	2
		peg protrusion	2
H	Lower impingement chamber	Conical flask	250 ml
		ground-glass inlet socket	24/29

Dimensions in millimetres, unless otherwise stated.



should be minimised and typically would not be greater than 10. Dismantle the apparatus. Wash the inner surface of the inlet tube to the lower impingement chamber and its outer surface that projects into the chamber with a suitable solvent, collecting the washings in the lower impingement chamber. Determine the content of active substance in this solution. Calculate the amount of active substance collected in the lower impingement chamber per discharge and express the results as a percentage of the dose stated on the label.

Apparatus B. Andersen Cascade impactor

The Andersen 1 ACFM non-viable cascade impactor consists of 8 stages together with a final filter. Material of construction may be aluminium, stainless steel or other suitable material. The stages are clamped together and sealed with O-rings. Critical dimensions applied by the manufacturer of apparatus B are provided in Table 3. In use, some occlusion and wear of holes will occur. In-use mensuration tolerances need to be justified. In the configuration used for pressurised inhalers (Fig. 4) the entry cone of the impactor is connected to an induction port (Fig. 9). A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port.

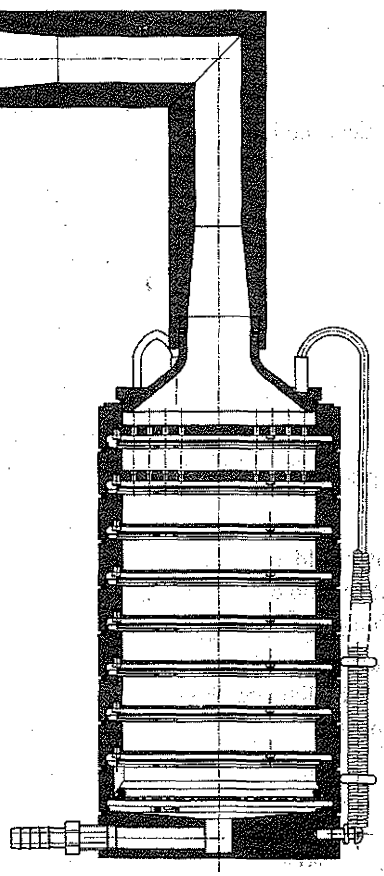


Fig. 4: Apparatus B. Andersen cascade impactor used for *pressurised inhalers*

In the configuration for powder inhalers, a pre-separator is placed above the top stage to collect large masses of non-respirable powder. It is connected to the induction port as shown in Fig. 5. To accommodate high flow rates through the impactor, the outlet nipple, used to connect the impactor to the vacuum system is enlarged to have an internal diameter of greater than or equal to 8 mm.

Table 3 - Critical dimensions for Apparatus B

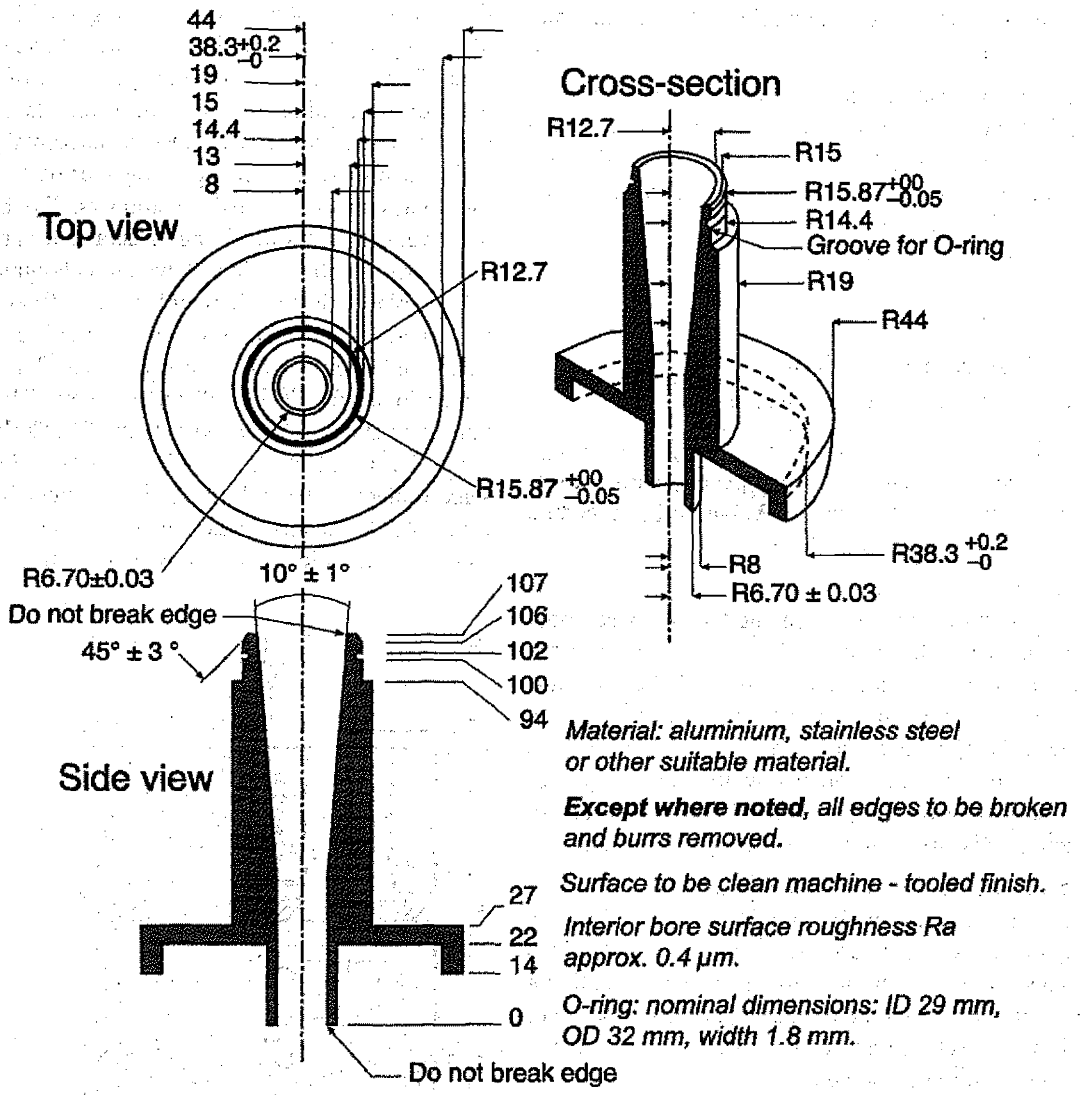
Description	Number	Dimension (mm)
Stage 0 nozzle diameter	96	2.55 ± 0.025
Stage 1 nozzle diameter	96	1.89 ± 0.025
Stage 2 nozzle diameter	400	0.914 ± 0.0127
Stage 3 nozzle diameter	400	0.711 ± 0.0127
Stage 4 nozzle diameter	400	0.533 ± 0.0127
Stage 5 nozzle diameter	400	0.343 ± 0.0127
Stage 6 nozzle diameter	400	0.254 ± 0.0127
Stage 7 nozzle diameter	201	0.254 ± 0.0127

Procedure for pressurised inhalers

Assemble the Andersen impactor with a suitable filter in place. Ensure that the system is airtight. In that respect, follow the manufacturer's instructions. Place a suitable mouthpiece adapter in position at the end of the induction port so that the mouthpiece end of the actuator, when inserted, lines up along the horizontal axis of the induction port and the inhaler unit is positioned in the same orientation as the intended use. Connect a suitable pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 28.3 litres per minute (± 5 per cent). Switch off the pump.

Unless otherwise prescribed in the patient instructions shake the inhaler for 5 seconds and discharge one delivery to waste. Switch on the pump to the apparatus, locate the mouthpiece end of the actuator in the adapter and discharge the inverted inhaler into the apparatus, depressing the valve for a sufficient time to ensure complete discharge. Wait for 5 seconds before removing the assembled inhaler from the adapter. Repeat the procedure. The number of discharges should be minimised and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of the fine particle dose. After the final discharge, wait for 5 seconds and then switch off the pump.

Dismantle the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove



(Dimensions in millimetres, unless otherwise stated)

Fig. 5: Connection of the induction port to the preseparator of the Andersen cascade impactor

the induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. Extract the active substance from the inner walls and the collection plate of each of the stages of the apparatus into aliquots of solvent.

Using a suitable method of analysis, determine the quantity of active substance contained in each of the aliquots of solvent.

Calculate the fine particle dose (see Calculations).

Procedure for powder inhalers

The aerodynamic cut-off diameters of the individual stages of this apparatus are currently not well-established at flow rates other than 28.3 litres per minute.

Users must justify and validate the use of the impactor in the chosen conditions, when flow rates different from 28.3 litres per minute are selected.

Assemble the Andersen impactor with the pre-separator and a suitable filter in place and ensure that the system is airtight. Depending on the product characteristics, the pre-separator may be omitted, where justified and authorised. Stages 6 and 7 may also be omitted at high flow rates, if justified. The pre-separator may be coated in the same way as the plates or may contain 10 ml of a suitable solvent. Connect the apparatus to a flow system according to the scheme specified in Fig. 10 and Table 6.

Unless otherwise defined, conduct the test at the flow rate, Q_{out}, used in the test for uniformity of delivered dose

drawing 4 litres of air from the mouthpiece of the inhaler and through the apparatus.

Connect a flow meter to the induction port. Use a flow meter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

Where, P_0 = Atmospheric pressure;
ΔP = Pressure drop over the meter.

Adjust the flow control valve to achieve steady flow through the system at the required rate, Q_{out} (± 5 per cent). Ensure that critical flow occurs in the flow control valve by the procedure described for Apparatus C. Switch off the pump.

Prepare the powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time, T (± 5 per cent). Repeat the discharge sequence. The number of discharges should be minimised and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the pre-separator, induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. Extract the active substance from the inner walls and the collection plate of each of the stages of the apparatus into aliquots of solvent.

Using a suitable method of analysis, determine the quantity of active substance contained in each of the aliquots of solvent.

Calculate the fine particle dose (see Calculations).

Apparatus C. Multi-stage liquid impinger

The multi-stage liquid impinger consists of impaction stages 1 (Pre-separator), 2, 3 and 4 and an integral filter stage (stage 5) (see Fig. 6/8). An impaction stage comprises an upper horizontal metal partition wall (B) through which a metal inlet jet tube (A) with its impaction plate (D) is protruding. A glass cylinder (E) with sampling port (F) forms the vertical wall of the stage, and a lower horizontal metal partition wall (G) through which the tube (H) connects to the next lower stage. The tube into stage 4 (U) ends in a multi-jet arrangement. The impaction plate (D) is secured in a metal frame (J) which is fastened by 2 wires (K) to a sleeve (L) secured on the jet tube. The horizontal face of the collection plate is perpendicular to the axis of the jet tube and centrally aligned. The upper surface of the impaction plate is slightly raised above the edge of the metal frame. A recess around the perimeter of the horizontal partition wall guides the position of the glass cylinder. The glass cylinders are sealed against the horizontal partition walls with gaskets (M) and clamped together by 6 bolts (N). The sampling ports are sealed by stoppers. The bottom-side of the lower partition wall of Stage 4 has a concentrical protrusion fitted with a rubber O-ring (P) which seals against the edge of a filter placed in the filter holder. The filter holder (R) is constructed as a basin with a concentrical recess in which a perforated filter support (S) is flush-fitted. The filter holder is dimensioned for 76 mm diameter filters. The assembly of impaction stages is clamped onto the filter holder by 2 snap-locks (T). Connect an induction port (see Fig. 9) onto the stage 1 inlet jet tube of the impinger. A rubber O-ring on the jet tube provides an airtight connection to the induction port. A suitable mouthpiece adapter is used to provide an airtight

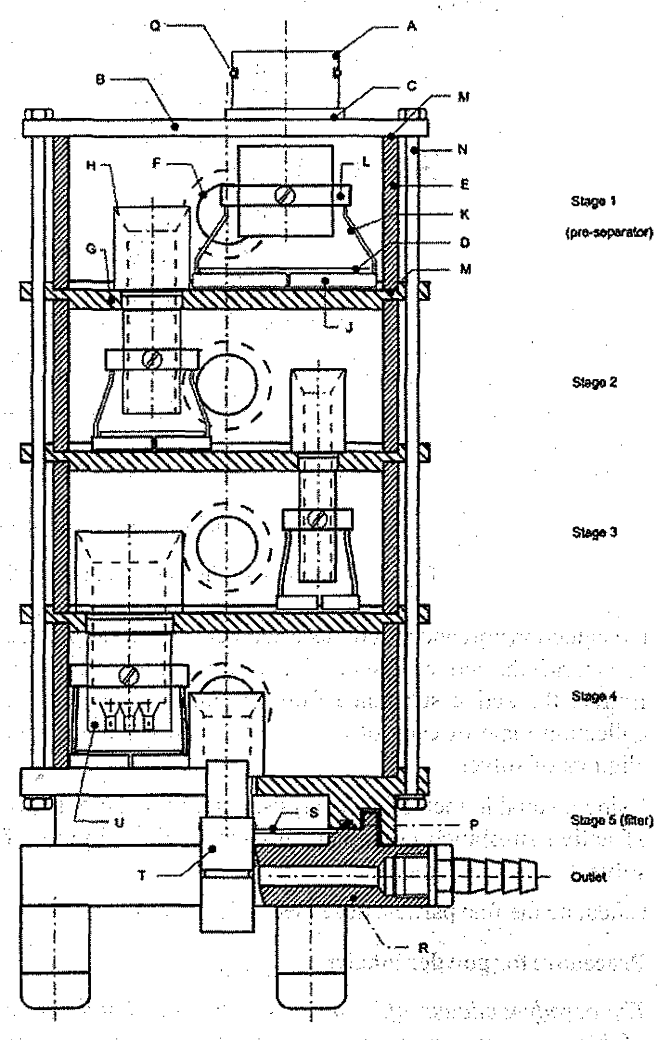


Fig. 6: Multi-stage liquid impinger

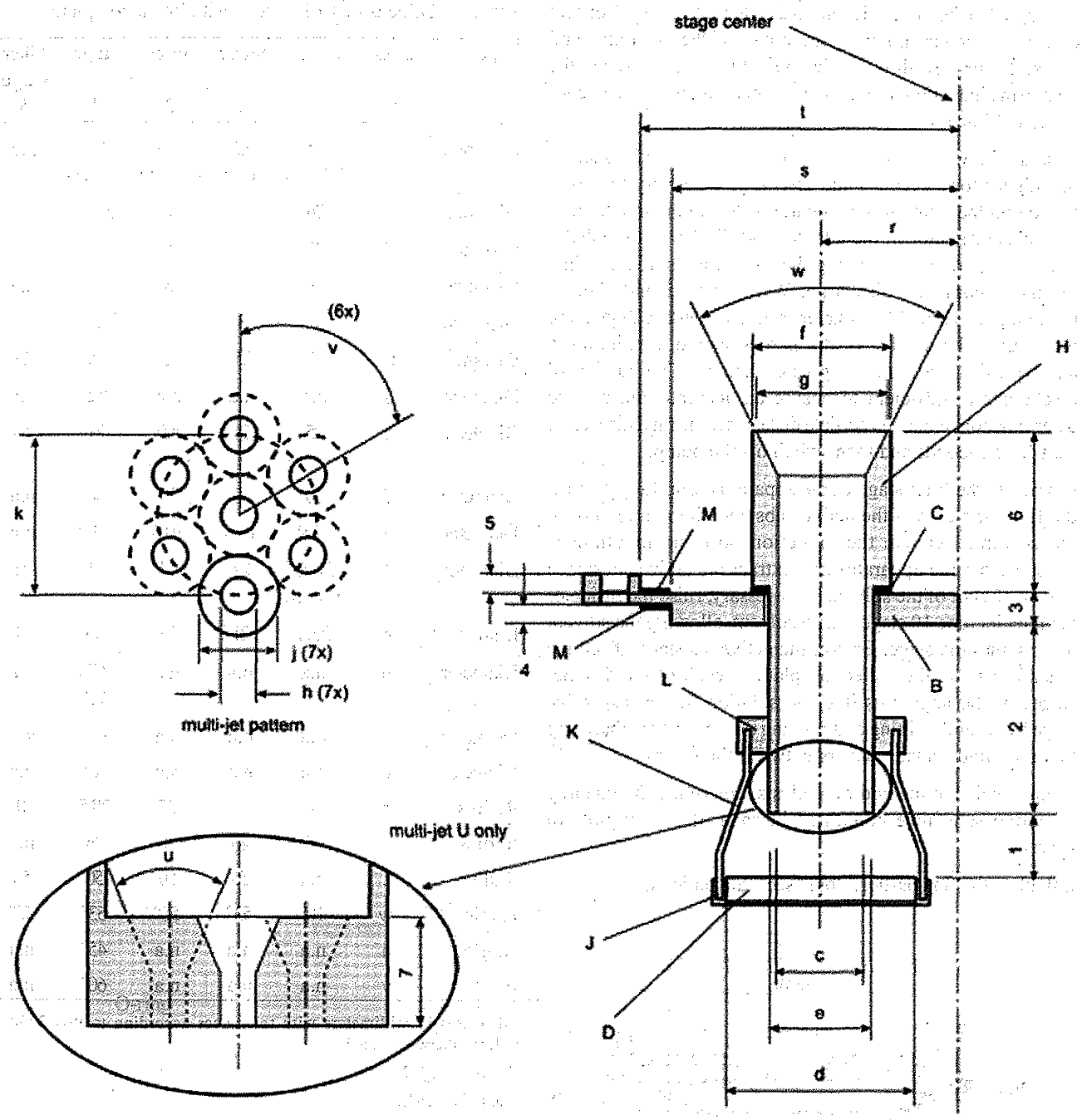


Fig. 7: Details of jet tube and impaction plate. Inserts show end of multi-jet tube U leading to stage 4. (Numbers and lowercase letters refer to Table 5 and uppercase letters refer to Fig. 6)

seal between the inhaler and the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port.

Procedure for pressurised inhalers

Dispense 20 ml of a solvent, capable of dissolving the active substance into each of stages 1 to 4 and replace the stoppers. Tilt the apparatus to wet the stoppers, thereby neutralising electrostatic charge. Place a suitable filter capable of quantitatively collecting the active substance in stage 5 and assemble the apparatus. Place a suitable mouthpiece adapter in position at the end of the induction port so that the mouthpiece end of the actuator, when inserted, lines up along the horizontal axis of the induction port and the inhaler is

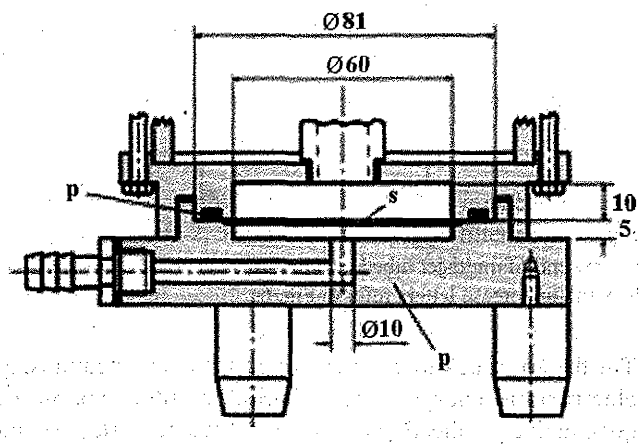
positioned in the same orientation as intended for use. Connect a suitable vacuum pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 30 litres per minute (± 5 per cent). Switch off the pump.

Unless otherwise prescribed in the patient instructions shake the inhaler for 5 seconds and discharge 1 delivery to waste. Switch on the pump to the apparatus, locate the mouthpiece end of the actuator in the adapter and discharge the inhaler into the apparatus, depressing the valve for a sufficient time to ensure complete discharge. Wait for 5 seconds before removing the assembled inhaler from the adapter. Repeat the procedure. The number of discharges should be minimised and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of the fine particle dose. After the final discharge, wait for 5 seconds and then switch off the pump.

Dismantle the filter stage of the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. If necessary, rinse the inside of the inlet jet tube 10 stage 1 with solvent, allowing the solvent to flow into the stage. Extract the active substance from the inner walls and the collection plate of each of the 4 upper stages of the apparatus into the solution in the respective stage by carefully tilting and rotating the apparatus, observing that no liquid transfer occurs between the stages.

Using a suitable method of analysis, determine the quantity of active substance contained in each of the aliquots of solvent.

Calculate the fine particle dose (sec Calculations).



(Dimensions in millimetres, unless otherwise stated)

Fig. 8: Details of the filter stage (stage 5). Numbers refer to dimensions (≥ Ø = diameter). Uppercase letters refer to Table 4.

Table 5 – Dimensions[1] of jet tube with impaction plate

Type	Code[2]	Stage 1	Stage 2	Stage 3	Stage 4	Filter stage 5
Distance	1	9.5 (-.0+.5)	5.5 (-.0+.5)	4.0 (-.0+.5)	6.0 (-.0+.5)	n.a
Distance	2	26	31	33	30.5	0
Distance	3	8	5	5	5	5
Distance	4	3	3	3	3	n.a
Distance	5	0	3	3	3	3
Distance	6[3]	20	25	25	25	25
Distance	7	n.a	n.a	n.a	8.5	n.a
Diameter	c	25	14	8.0 (±.1)	21	14
Diameter	d	50	30	20	30	n.a
Diameter	e	27.9	16.5	10.5	23.9	n.a
Diameter	f	31.75 (.0+.5)	22	14	31	22
Diameter	g	25.4	21	13	30	21
Diameter	h	n.a	n.a	n.a	2.70 (±.5)	n.a
Diameter	j	n.a	n.a	n.a	6.3	n.a
Diameter	k	n.a	n.a	n.a	12.6	n.a
Radius[4]	r	16	22	27	28.5	0
Radius	s	46	46	46	46	n.a
Radius	t	n.a	50	50	50	50
Angle	w	10°	53°	53°	53°	53°
Angle	u	n.a	n.a	n.a	45°	n.a
Angle	v	n.a	n.a	n.a	60°	n.a

[1]Measures in millimeters with tolerances according to ISO 2768-m unless otherwise stated,

[2]Refer to Fig. 7

[3]Including gasket

[4]Relative centreline of stage compartment.

n.a. = not applicable

Procedure for powder inhalers

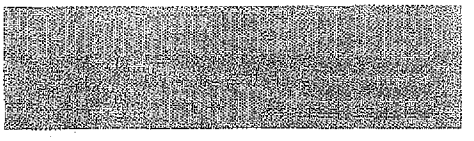
Place a suitable low resistance filter capable of quantitatively collecting the active substance in stage 5 and assemble the apparatus. Connect the apparatus to a flow system according to the scheme specified in Fig. 10 and Table 6. Unless otherwise defined, conduct the test at the flow rate, Q_{out}, used in the test for uniformity of delivered dose, drawing 4 litre of air from the mouthpiece of the inhaler and through the apparatus.

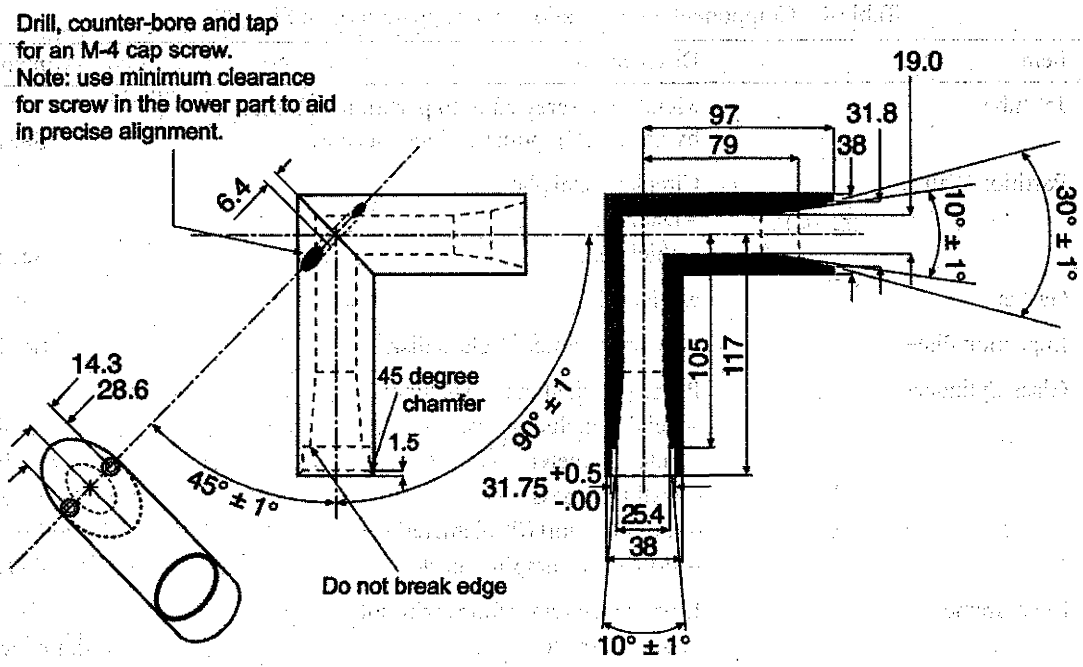
Table 4 – Components specification in for apparatus C in Fig. 6/8

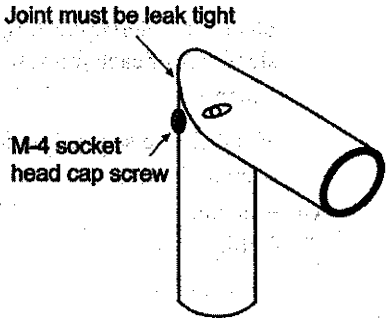
Code*	Item	Description	Dimensions**
A, H	Jet tube	Metal tube screwed onto partition wall sealed by gasket (C), polished inner surface	See Fig. 7
B,G	Partition Wall	Circular metal plate	
		– Diameter	120
		– thickness	See Fig. 7
C	Gasket	e.g. PTFE	to fit jet tube
D	Impaction Plate	Porosity 0 sintered-glass disk	See Fig. 7
E	Glass Cylinder	Plane polished cut glass tube:	
		– height, including gaskets	46
		– outer diameter	100
		– wall thickness	3.5
		– sampling port (F) diameter	18
		– stopper in sampling port	ISO 24/25
J	Metal frame	L-profiled circular frame with slit	
		– inner diameter	to fit impaction plate
		– height	4
		– thickness of horizontal section	0.5
		– thickness of vertical section	2
K	Wire	Steel wire interconnecting metal frame and sleeve (2 for each frame)	
		– diameter	1
L	Sleeve	Metal sleeve secured on jet tube by screw	
		– inner diameter	to fit jet tube
		– height	6
		– thickness	5
M	Gasket	e.g. silicone	to fit glass cylinder
N	Bolt	Metal bolt with nut (6 pairs)	
		– length	205
		– diameter	4
P	O-ring	Rubber O-ring	
		– diameter x thickness	66.34 x 2.62
Q	O-ring	Rubber O-ring	
		– diameter x thickness	29.1 x 1.6
R	Filter holder	Metal housing with stand and outlet	See Fig. 8
S	Filter support	Perforated sheet metal	
		– diameter	65
		– hole diameter	3
		– distance between holes (centre-points)	4
T	Snap-locks		
U	Multi-jet tube	Jet tube (H) ending in multi-jet arrangement.	See inserts Fig. 7

* Refers to Fig. 6.

** Measures in millimeters with tolerances according to iso 2768-m unless otherwise stated.







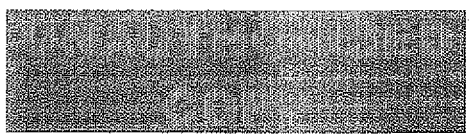
Isometric view of induction port

Note:

1. Material may be aluminium, stainless steel or other suitable material.
2. Machine from 38 mm bar stock.
3. Bore 19 mm hole through bar.
4. Cut tube to exact 45^0 as shown.
5. The inner bores and tapers should be smooth - surface roughness Ra approx. 0.4µm.
6. Mill joining cads of stock to provide a liquid tight leak-free seal.
7. Set up a holding fixture for aligning the inner 19 mm bore and for drilling and tapping M4 × 0.7 threads. There must be virtually no mismatch of the inner bores in the miter joint.

(Dimensions in millimetres, unless otherwise stated)

Fig.9: Induction port



Connect a flow meter to the induction port. Use a flow meter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

Where, P_0 = Atmospheric pressure;
ΔP = Pressure drop over the meter.

Adjust the flow control valve to achieve steady flow through the system at the required rate, Q_{out} (± 5 per cent). Switch off the pump. Ensure that critical flow occurs in the flow control valve by the following procedure.

With the inhaler in place and the test flow rate established, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Fig. 10). A ratio P3/P2 of less than or equal to 0.5 indicates critical flow. Switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

Dispense 20 ml of a solvent, capable of dissolving the active substance into each of the 4 upper stages of the apparatus and replace the Stoppers. Tilt the apparatus to wet the stoppers, thereby neutralising electrostatic charge. Place a suitable mouthpiece adapter in position at the end of the induction port.

Prepare the powder inhaler for use according to patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the

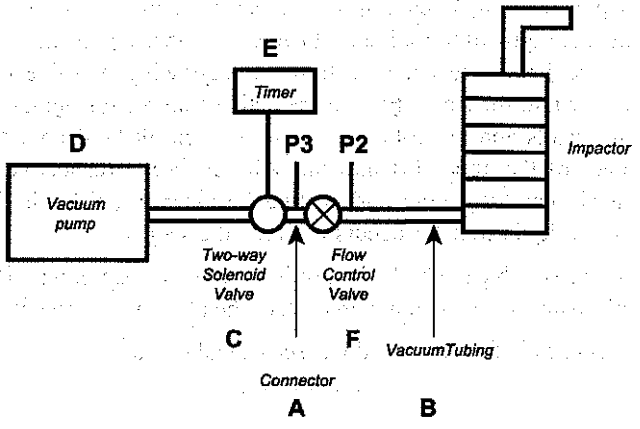


Fig. 10: Experimental set-up for testing powder inhalers

Table 6 – Component specification for set-up in Fig. 10

Code	Item	Description
A	Connector	ID ≥ 8 mm, e.g., short metal coupling with low-diameter branch to P3.
B	Vacuum tubing	A length of suitable tubing having an ID ≥ 8 mm and an internal volume of 25 ± 5 ml.
C	2-way solenoid valve	A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with ID ≥ 8 mm and an opening time ≤ 100 ms. (e.g. type 256 - A08), Burkert GmbH, D-74653 Ingelfingen), or equivalent.
D	Vacuum pump	Pump must be capable of drawing the required flow rate through the assembled apparatus with the powder inhaler in the mouthpiece adapter (e.g. product type 1023, 1423 or 2565, Gast Manufacturing Inc., Benton Harbor, MI 49022), or equivalent. Connect the pump to the 2-way solenoid valve using short and / or wide (ID ≥ 10 mm) vacuum tubing and connectors to minimize pump capacity requirements.
G	Timer	Timer capable to drive the 2-way solenoid valve for the required duration (e.g. type G814, RS components International, Corby, NN17 9RS, UK), or equivalent.
P2, P3	Pressure measurements	Determine under steady-state flow condition with an absolute pressure transducer.
F	Flow control valve	Adjustable regulating valve with maximum Cv, ≥ 1, (e.g. type 8FV12LNSS, Parker Hannifin plc., Barnstaple, EX311 NP, UK), or equivalent.

mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time, T (± 5 per cent). Repeat the procedure. The number of discharges should be minimised and typically would not greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the filter stage of the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. If necessary, rinse the inside of the inlet jet tube to stage 1 with solvent, allowing the solvent to flow into the stage. Extract the active substance from the inner walls and the collection plate of each of the 4 upper stages of the apparatus into the solution in the respective stage by carefully tilting and rotating the apparatus, observing that no liquid transfer occurs between the stages.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent.

Calculate the fine particle dose (see Calculations).

Apparatus D. Cascade impactor with 7 Stages and a Micro orifice collector (MOC)

Appararus D is a cascade impactor with 7 stages and a micro-orifice collector (MOC). Over the flow rate range of 30 litre per minutes to 100 litres per minutes the 50 per cent efficiency cut-off diameters (D_{50} values) range between 0.24 µm to 11.7 µm, evenly spaced on a logarithmic scale. In this flow range, there are always at least 5 stages with D_{50} values between 0.5 µm and 6.5 µm. The collection efficiency curves for each stage are sharp and minimise overlap between stages.

Material of construction may be aluminium, stainless steel or other suitable material.

The impactor configuration has removable impaction cups with all the cups in one plane (Fig. 11/14). There are 3 main sections to the impactor; the bottom frame that holds the impaction cups, the seal body that holds the jets and the lid that contains the interstage passageways (Fig. 11/12). Multiple nozzles are used at all but the first stage (Fig. 13). The flow passes through the impactor in a saw-tooth pattern.

Critical dimensions are provided in Table 7.

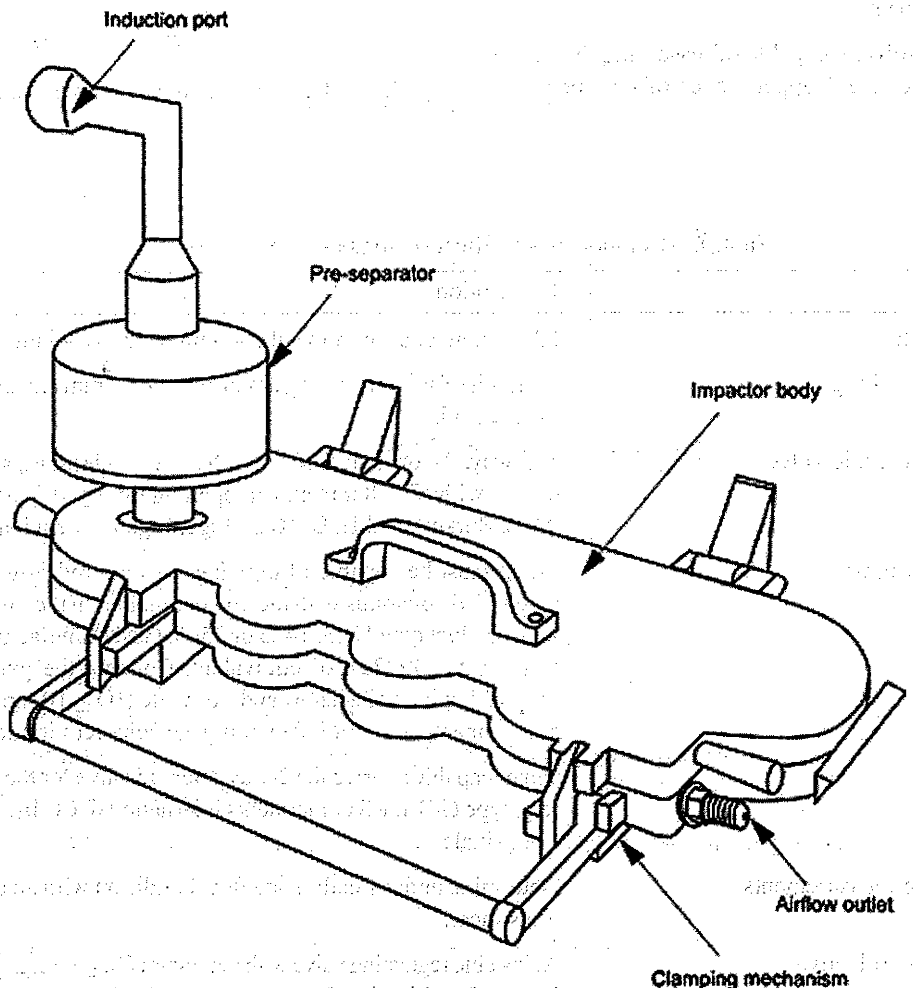


Fig. 11: Apparatus D (Shown with the pre-separator in place)

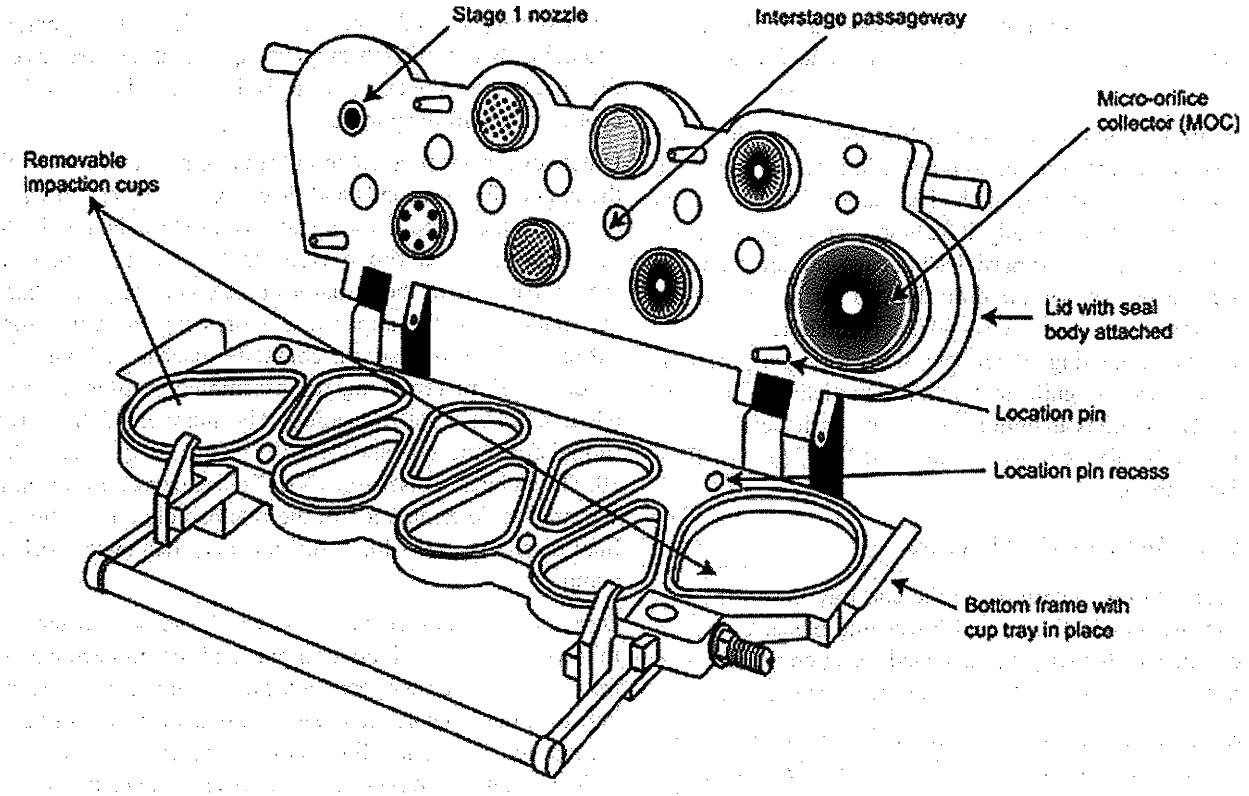


Fig. 12: Apparatus D showing component parts

Table 7– Critical dimensions for apparatus D

Description	Dimension (mm)
Pre-separator (dimension a - see Fig. 15)	12.8 ± 0.05
Stage 1* Nozzle diameter	14.3 ± 0.05
Stage 2* Nozzle diameter	4.88 ± 0.04
Stage 3* Nozzle diameter	2.185 ± 0.02
Stage 4* Nozzle diameter	1.207 ± 0.01
Stage 5* Nozzle diameter	0.608 ± 0.01
Stage 6* Nozzle diameter	0.323 ± 0.01
Stage 7* Nozzle diameter	0.206 ± 0.01
MOC*	Approx. 0.070
Cup depth (dimension b- see Fig. 14)	14.625 ± 0.10
Collection cup surface roughness (Ra)	0.5 – 2 µm
Stage 1 nozzle to seal body distance** - dimension C	0 ± 1.18
Stage 2 nozzle to seal body distance** - dimension C	5.236 ± 0.736
Stage 3 nozzle to seal body distance** - dimension C	8.445 ± 0.410
Stage 4 nozzle to seal body distance** - dimension C	11.379 ± 0.273
Stage 5 nozzle to seal body distance** - dimension C	13.176 ± 0.341
Stage 6 nozzle to seal body distance** - dimension C	13.999 ± 0.071
Stage 7 nozzle to seal body distance** - dimension C	14.000 ± 0.071
MOC nozzle to seal body distance** - dimension C	14.429 to 14.571

*See Fig. 13,

**See Fig. 14.

In routine operation, the seal body and lid are held together as a single assembly. The impaction cups are accessible when this assembly is opened at the end of an inhaler test. The cups are held in a support tray, so that all cups can be removed from the impactor simultaneously by lifting out the tray.

An induction port with internal dimensions (relevant to the airflow path) defined in Fig. 9 connects to the impactor inlet. A pre-separator can be added when required, typically with powder inhalers, and connects between the induction port

and the impactor. A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port.

Apparatus D contains a terminal Micro-Orifice Collector (MOC) that for most formulations will eliminate the need for a final filter as determined by method validation. The MOC is an impactor plate with nominally 4032 holes, each approximately 70 µm in diameter. Most particles not captured on stage 7 of the impactor will be captured on the cup surface below the MOC. For impactors operated at 60 liter per minutes, the MOC is capable of collecting 80 per cent of 0.14 µm particles. For formulations with a significant fraction of particles not captured by the MOC, there is an optional filter holder that can replace the MOC or be placed downstream of the MOC (a glass fibre filter is suitable).

Procedure for pressurised inhalers

Place cups into the apertures in the cup tray. Insert the cup tray into the bottom frame, and lower into place. Close the impactor lid with the seal body attached and operate the handle to lock the impactor together so that the system is airtight.

Connect an induction port with internal dimensions defined in Fig. 9 to the impactor inlet. Place a suitable mouthpiece adapter in position at the end of the induction port so that the mouthpiece end of the actuator, when inserted, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use. Connect a suitable pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 30 liter per minutes (± 5 per cent). Switch off the pump.

Unless otherwise prescribed in the patient instructions shake the inhaler for 5 seconds and discharge 1 delivery to waste. Switch on the pump to the apparatus. Prepare the inhaler for use according to the patient instructions, locate the mouthpiece end of the actuator in the adapter and discharge the inhaler into the apparatus, depressing the valve for a sufficient time to ensure a complete discharge. Wait for 5 seconds before removing the assembled inhaler from the adapter. Repeat the procedure. The number of discharges should be minimised, and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of the fine panicle dose. After the final discharge, wait for 5 seconds and then switch off the pump.

Dismantle the apparatus and recover the active substance as follows: remove the induction port and mouthpiece adapter from the apparatus and recover the deposited active substance into an aliquot of solvent. Open the impactor by releasing the handle and lifting the lid. Remove the cup tray, with the collection cups, and recover the active substance in each cup into an aliquot of solvent.

Using a suitable method of analysis, determine the quantity of active substance contained in each of the aliquots of solvent.

Calculate the fine panicle dose (see Calculations).

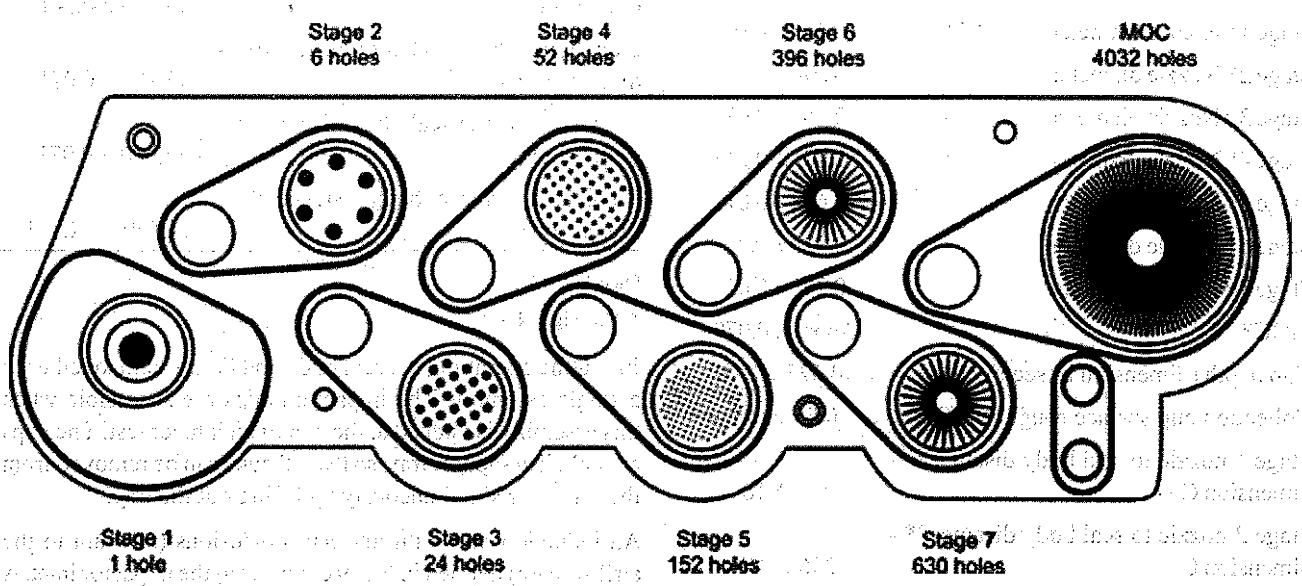


Fig. 13: Apparatus D: nozzle configuration

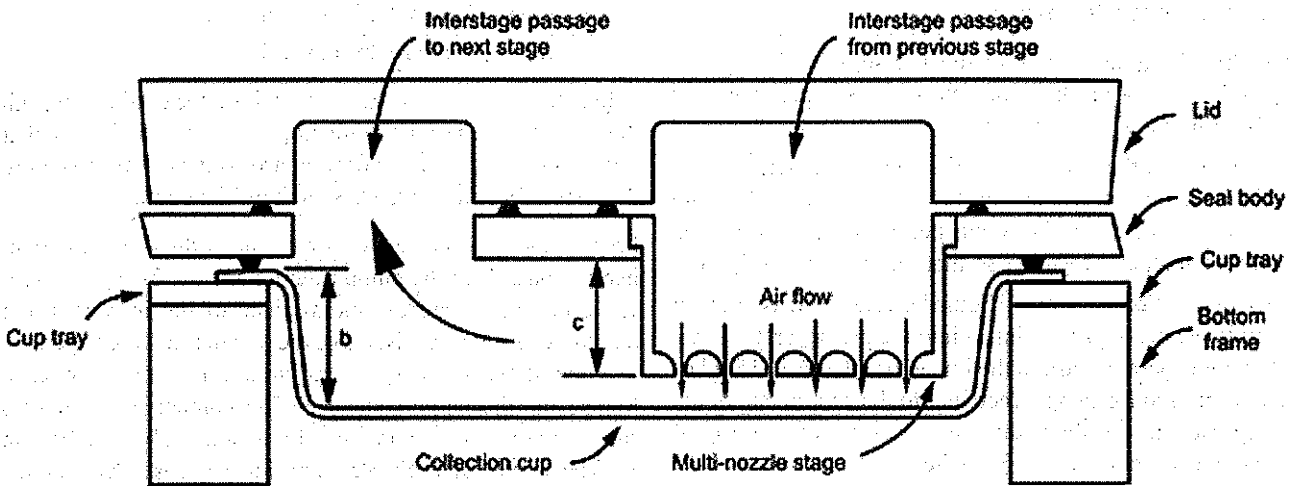


Fig. 14: Apparatus D: configuration of interstage passageways

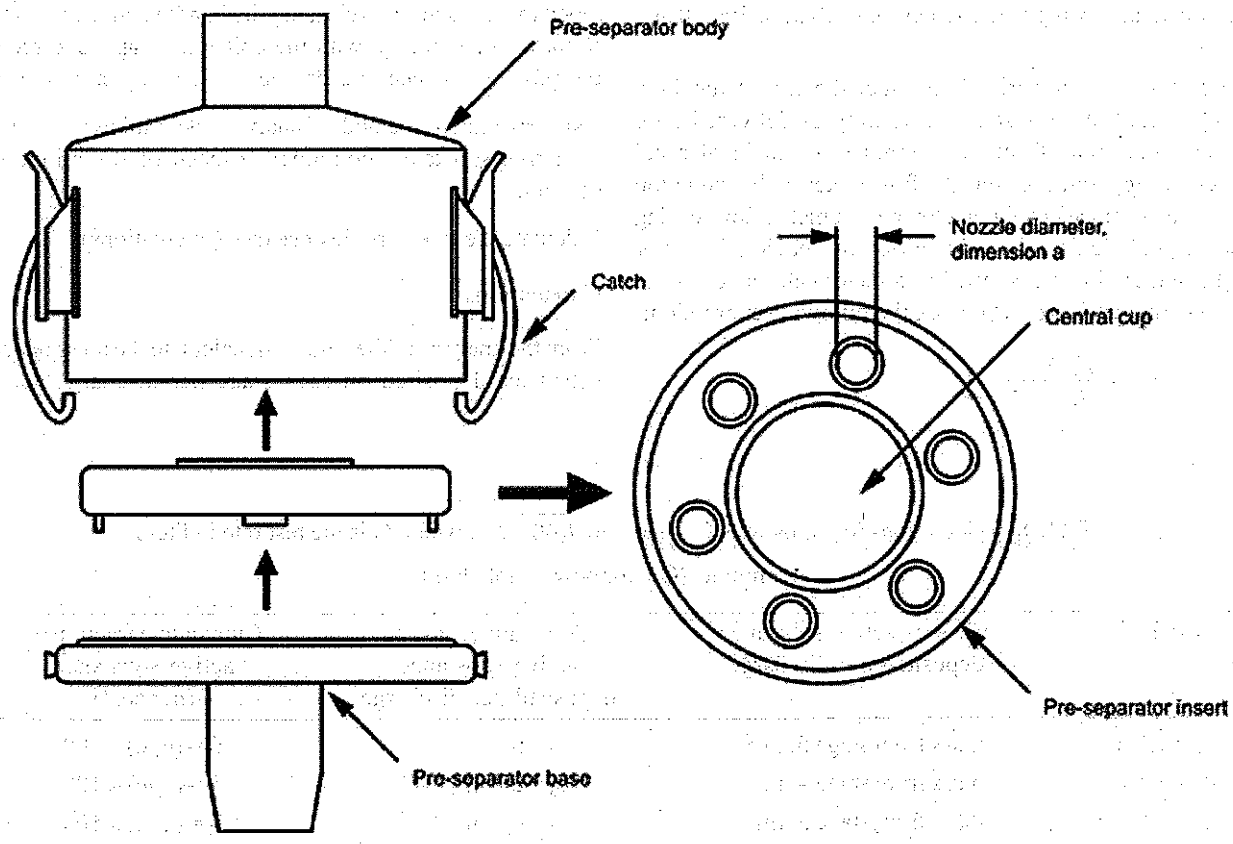


Fig. 15: Apparatus D: Pre-seperator configuration

Procedure for powder inhalers

Assemble the apparatus with the pre-separator (Fig. 15) Depending on the product characteristics, the pre-separator may be omitted, where justified.

Place cups into the apertures in the cup tray. Insert the cup tray into the bottom frame, and lower into place. Close the impactor lid with the seal body attached and operate the handle to lock the impactor together so that the system is airtight.

When used, the pre-separator should be assembled as follows: assemble the pre-separator insert into the pre-separator base. Fit the pre-separator base to the impactor inlet. Add 15 ml of the solvent used for sample recovery to the central cup of the pre-separator insert. Place the pre-separator body on top of this assembly and close the 2 catches.

Connect an induction port with internal dimensions defined in Fig. 9 to the impactor inlet or pre-separator inlet. Place a suitable mouthpiece adapter in position at the end of the induction port so that the mouthpiece end of the inhaler, when inserted, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use. Connect the apparatus to a flow system according to the scheme specified in Fig. 10 and Table 6.

Unless otherwise prescribed, conduct the test at the flow rate, Q_{out}, used in the test for uniformity of delivered dose drawing 4 liter of air from the mouthpiece of the inhaler and through the apparatus. Connect a flow meter to the induction port. Use a flow meter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

where, P_0 = Atmospheric pressure;

ΔP = Pressure drop over the meter.

Adjust the flow control valve to achieve steady flow through the system at the required rate, Q_{out} (± 5 per cent). Ensure that critical flow occurs in the flow control valve by the procedure described for Apparatus D. Switch off the pump.

Prepare the powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time, T (± 5 per cent). Repeat the discharge sequence. The number of discharges should be minimised and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the apparatus and recover the active substance as follows: remove the induction port and mouthpiece adapter from the pre-separator, when used, and recover the deposited active substance into an aliquot of solvent. When used, remove the pre-separator from the impactor, being careful to avoid spilling the cup liquid into the impactor. Recover the active substance from the pre-separator.

Open the impactor by releasing the handle and lifting the lid. Remove the cup tray, with the collection cups, and recover the active substance in each cup into an aliquot of solvent.

Using a suitable method of analysis, determine the quantity of active substance contained in each of the aliquots of solvent.

Calculate the fine particle dose (see Calculations).

Calculations

From the analysis of the solutions, calculate the mass of active substance deposited on each stage per discharge and the

Table 8. Calculations for Apparatus C. Use $q = \sqrt{(60/Q)}$, where Q is the test rate in liters per minute (Q_{out} for powder inhalers)

Cut-off diameter (µm)	Mass of active substance deposited per discharge	Cumulative mass of active substance deposited per discharge	Cumulative fraction of active substance (per cent)
$d_4 = 1.7 \times q$	mass from stage 5, m_5*	$c_4 = m_5$	$f_4 = (c_4/c) \times 100$
$d_3 = 3.1 \times q$	mass from stage 4, m_4	$c_3 = c_4 + m_4$	$f_3 = (c_3/c) \times 100$
$d_2 = 6.8 \times q$	mass from stage 3, m_3	$c_2 = c_3 + m_3$	$f_2 = (c_2/c) \times 100$
	mass from stage 2, m_2	$c = c_2 + m_2$	100

*Stage 5 is the filter stage.

Table 9 – Calculations for Apparatus B when used at a flow rate of 28.3 litres per minute

Cut-off diameter (μm)	Mass of active substance deposited per discharge	Cumulative mass of active substance deposited per discharge	Cumulative fraction of active substance (per cent)
$d_7 = 0.4$	mass from stage 8, m_8	$c_7 = m_8$	$f_7 = (c_7/c) \times 100$
$d_6 = 0.7$	mass from stage 7, m_7	$c_6 = c_7 + m_7$	$f_6 = (c_6/c) \times 100$
$d_5 = 1.1$	mass from stage 6, m_6	$c_5 = c_6 + m_6$	$f_5 = (c_5/c) \times 100$
$d_4 = 2.1$	mass from stage 5, m_5	$c_4 = c_5 + m_5$	$f_4 = (c_4/c) \times 100$
$d_3 = 3.3$	mass from stage 4, m_4	$c_3 = c_4 + m_4$	$f_3 = (c_3/c) \times 100$
$d_2 = 4.7$	mass from stage 3, m_3	$c_2 = c_3 + m_3$	$f_2 = (c_2/c) \times 100$
$d_1 = 5.8$	mass from stage 2, m_2	$c_1 = c_2 + m_2$	$f_1 = (c_1/c) \times 100$
$d_0 = 9.0$	mass from stage 1, m_1	$c_0 = c_1 + m_1$	$f_0 = (c_0/c) \times 100$
	mass from stage 0, m_0	$c = c_0 + m_0$	100

Table 10 – Calculations for Apparatus D. Use $q = (60/Q)^x$, where Q is the test flow rate in liters per minute, and x is listed in the table

Cut-off diameter (μm)	x	Mass of active substance deposited per discharge deposited per discharge	Cumulative mass of active substance (per cent)	Cumulative fraction of active substance
$d_7 = 0.34 \times q$	0.67	mass from MOC or terminal filter, m_8	$c_7 = m_8$	$f_7 = (c_7/c) \times 100$
$d_6 = 0.55 \times q$	0.60	mass from stage 7, m_7	$c_6 = c_7 + m_7$	$f_6 = (c_6/c) \times 100$
$d_5 = 0.94 \times q$	0.53	mass from stage 6, m_6	$c_5 = c_6 + m_6$	$f_5 = (c_5/c) \times 100$
$d_4 = 1.66 \times q$	0.47	mass from stage 5, m_5	$c_4 = c_5 + m_5$	$f_4 = (c_4/c) \times 100$
$d_3 = 2.82 \times q$	0.50	mass from stage 4, m_4	$c_3 = c_4 + m_4$	$f_3 = (c_3/c) \times 100$
$d_2 = 4.46 \times q$	0.52	mass from stage 3, m_3	$c_2 = c_3 + m_3$	$f_2 = (c_2/c) \times 100$
$d_1 = 8.06 \times q$	0.54	mass from stage 2, m_2	$c_1 = c_2 + m_2$	$f_1 = (c_1/c) \times 100$
		mass from stage 1, m_1	$c = c_1 + m_1$	100

mass of active substance per discharge deposited in the induction port, mouthpiece adapter and when used, the pre-separator.

Starting at the final collection site (filter or MOC), derive a table of cumulative mass versus cut-off diameter of the respective stage (see Tables 8 for Apparatus C, 9 for Apparatus B, 10 for Apparatus D). Calculate by interpolation the mass of the active substance less than 5 μm. This is the Fine Particle Dose (FPD).

If necessary, and where appropriate (e.g., where there is a log-normal distribution), plot the cumulative fraction of active substance versus cut-off diameter (see Tables 8/10) on log probability paper, and use this plot to determine values for the Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) as appropriate. Appropriate computational methods may also be used.

Preparations for Nebulisation: Characterisation

Products used for nebulisation and intended for pulmonary delivery are characterised using the following tests:

- Active substance delivery rate and total active substance delivered;
- Aerodynamic assessment of nebulised aerosols.

These tests standardise the approach given to the assessment of the dose that would be delivered to a patient but are not intended to provide assessment of the nebuliser device itself.

The mass- rather than the number-weighted size distribution is more appropriate to evaluate product performance. Indeed,

active substance mass as a function of aerodynamic diameter is more indicative of therapeutic effect within the respiratory tract.

Active substance delivery rate and Total active substance delivered

These tests are performed to assess the rate of delivery to the patient and the total active substance delivered to the patient, using standardised conditions of volumetric flow rate. It is essential that breath-enhanced and breath-actuated nebulisers be evaluated by a breathing simulator, as the output of these types of device is highly dependent on inhalation flow rate. The methodology below describes the use of a standard breathing pattern defined for adults. Should a particular product for nebulisation only be indicated for paediatric (i.e. neonate, infant or child) use, and then paediatric breathing pattern(s) must be used. Breathing patterns are used, rather than continuous flow rates, to provide a more appropriate measure of the mass of active substance that would be delivered to patients.

Active substance delivery rate and total active substance delivered are appropriate characteristics because they allow the mass delivered to be characterised in a standard way regardless of the nebuliser used. Accordingly, the test methodology described below allows that the mass of active substance delivered in the 1st period (typically 1 minute) is measured (consequently giving an assessment of active substance delivery rate) as well as capturing the total mass of active substance delivered.

Apparatus

Breathing simulator

A commercially available breathing simulator, which is able to generate the breathing profiles specified in Table 11, is used for the test. The breathing profile indicated for adults is used unless the medicinal product is specifically intended for use in paediatrics, where alternate patterns should be used, as indicated in Table 11.

Table 11 – Breathing simulator specifications

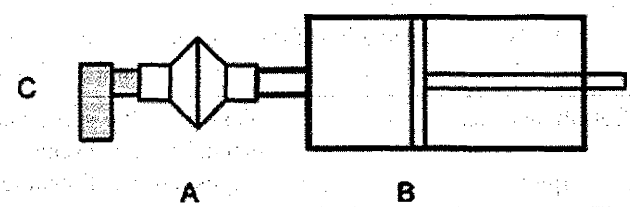
Item	Specification			
	Adult	Neonate	Infant	Child
Tidal volume	500 ml	25 ml	50 ml	155 ml
Frequency	15 cycles per minutes	40 cycles per minutes	30 cycles per minutes	25 cycles per minutes
Waveform	sinusoidal	sinusoidal	sinusoidal	sinusoidal
Inhalation/ exhalation ratio	1:1	1:3	1:3	1:2

Filter system

A suitably validated low-resistance filter, capable of quantitatively collecting the aerosol and enabling recovery of the active substance with an appropriate solvent, is used for the test. The dead volume of the filter casing does not exceed 10 per cent of the tidal volume used in the breath simulation.

Method

Attach the filter (contained in the filter holder) (A) to the breath simulator (B) according to Fig. 16 Fill the nebuliser (C) with the volume of the medicinal product as specified in the patient instructions. Attach the mouthpiece of the nebuliser to the inhalation filter using a mouthpiece adapter if required, ensuring that connections are airtight. Make sure the nebuliser is positioned in the same orientation as intended for use; this may require tilting the breathing simulator and filter holder. Set the breathing simulator to generate the specified breathing pattern.



A. Inhalation filter and filter holder
B. Breathing simulator
C. Nebuliser

Fig. 16: Experimental set-up for breathing simulator testing

Start the breathing simulator then, at the beginning of an inhalation cycle, start the nebuliser. Operate the nebuliser for a defined initial time period. The time chosen, usually 60 ± 1 second, must allow sufficient active substance deposition on the inhalation filter to allow quantitative analysis. If the quantity of active substance deposited on the inhalation filter in 60 seconds is in sufficient for this analysis, the length of the time interval for aerosol collection can be increased. If the filter is soaked with the preparation, this time can be decreased. At the end of this initial period, stop the nebuliser.

Place a fresh filter and filter holder in position and continue until nebulisation ceases. Interrupt nebulisation and exchange filters if necessary, to avoid filter saturation.

Results

Using a suitable method of analysis, determine the mass of active substance collected on the filters and filter holders during each time interval. Determine the active substance delivery rate by dividing the mass of active substance collected on the first inhalation filter by the time interval used for

collection. Determine the total mass of active substance delivered by summing the mass of active substance collected on all inhalation filters and filter holders.

Aerodynamic assessment of nebulised Aerosols

Nebulised products need to be size-characterised at flow rates lower than the range that is normally used for powder inhalers and metered-dose inhalers. A flow rate of 15 litre per minutes is recommended because this value represents a good approximation to the mid-inhalation flow rate achievable by a tidally breathing healthy adult (500 ml tidal volume). Although low-angle laser light scattering instruments (laser diffractometers) can provide rapid sae-distribution measurements of nebuliser-generated aerosols, these techniques do not detect the active substance; rather they measure the size distribution of the droplets irrespective of their content. This may not be a problem with homogeneous solutions, but can result in significant error if the product to be nebulised is a suspension, or if droplet evaporation is significant as can be the case with certain nebuliser types. Cascade impactors enable the aerosol to be characterised unambiguously in terms of the mass of active substance as a function of aerodynamic diameter. Laser diffraction may be used if validated against a cascade impaction method.

Apparatus D a cascade impactor, has been calibrated at 15 litres per minutes and is therefore used for this test. Determining mass balance in the same way as for powder inhalers and metered-dose inhalers is not straightforward, in that the dose is being captured as a continuous output, and hence is not included. As part of method development, recovery experiments must be performed to validate the method.

It is also recognised that the control of evaporation of droplets produced by nebulisers may be critical to avoid bias in the droplet size assessment process. Evaporation can be minimised by cooling the impactor to a temperature of about 5°, typically achieved by cooling the impactor in a refrigerator for about 90 minutes. Typically, at least after each day of use, the apparatus must be fully cleaned, including the inter-stage passageways, in view of the greater risk of corrosion caused by the condensation/accumulation of saline-containing droplets on inter-stage metalwork associated with cooling the impactor. It is recommended to dry all surfaces of the apparatus after each test, for example with compressed air. Note: the micro-orifice collector (MOC) should not be dried with compressed air.

Apparatus

A detailed description of Apparatus D and the induction port is contained in preparation for inhalation, and includes details of critical dimensions and the qualification process for the impactor (stage mensuration).

A back-up filter in addition to the micro-orifice collector (MOC) must be used to ensure quantitative recovery of active substance from the nebulised aerosol at the specified flow rate of 15 litres per minutes. The filter is located below the MOC (internal filter option) or a filter in holder, external to the impactor, is used to capture any fine droplets that pass beyond the last size fractionating stage.

A pre-separator is not used for testing nebuliser-generated aerosols.

Method validation

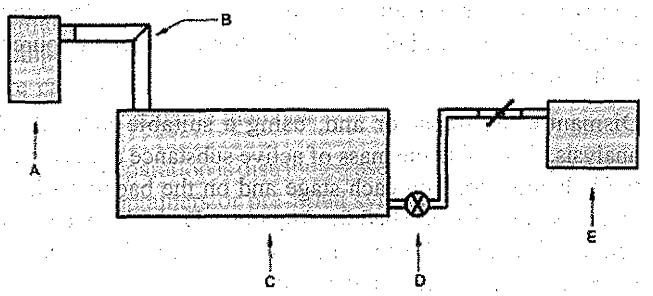
Impactor stage overloading

During method development and validation, it is important to confirm that the volume of liquid sampled from the nebuliser does not overload the impactor. Visual inspection of the collection surfaces on stages collecting most of the droplets may reveal streaking if overloading has occurred. This phenomenon is usually also associated with an increase in mass of active substance collected on the final stage and back-up filter. Reducing the sampling period (T_0) is the most effective way to avoid overloading in any given system, balancing overloading with analytical sensitivity.

Re-entrainment

Droplet bounce re-entrainment are less likely with nebuliser-produced droplets than with solid particles from inhalers and for that reason coating would not normally be required.

Method



A. Nebuliser
B. Induction port
C. Impactor(apparatus D)
D. Flow control valve
E. Vacuum source

Fig. 17: Apparatus D for measuring the size distribution of preparations for nebulisation

Pre-cool the assembled impactor and induction port in a refrigerator (set at about 5°) for not less than 90 minutes and start the determination within about 5 minutes of removal of

the impactor from the refrigerator. Other methods that maintain the impactor at a constant temperature (for example, use of a cooling cabinet) can also be employed when validated.

Set up the nebuliser with a supply of driving gas (usually air or oxygen), or use a compressor, at the pressure and flow rate specified by the manufacturer of the nebuliser. Take precautions to ensure that the gas supply line does not become detached from the nebuliser when under pressure. Fill the nebuliser with the volume of the medicinal product as specified in the patient instructions.

Remove the impactor from the refrigerator. Attach the induction port to the impactor, and connect the outlet of the impactor/ external filter to a vacuum source that is capable of drawing air through me system at 15 litres per minutes as specified in Fig. 17. Turn on the flow through the impactor.

Connect a flow meter, calibrated for the volumetric flow leaving the meter, to the induction port. Adjust the flow control valve located between the impactor and the vacuum source to achieve a steady flow through the system at 15 litres per minutes (± 5 per cent). Remove the flow meter.

Make sure the nebuliser is positioned in the same orientation as intended for use then attach the mouthpiece of the nebuliser to the induction port, using a mouthpiece adapter if required, ensuring that connections are airtight. Switch on the flow/ compressor for the nebuliser. Sample for a predetermined time (T_0). Once determined, this time (T_0) must be defined and used in the analytical method for a particular medicinal product to ensure that mass fraction data can be compared. At the end of the sampling period, switch off the driving gas flow/ compressor to the nebuliser, remove the nebuliser from the induction port and switch off the flow from the vacuum source to the impactor.

Dismantle the impactor and, using a suitable method of analysis, determine the mass of active substance collected in the induction port, on each stage and on the back-up filter/ external filter as described for Apparatus D. Add the mass of active substance collected in the MOC to that deposited on the back-up filter/external filter and treat as a single sample for the purpose of subsequent calculations.

Calculate the mass fraction ($F_{m,comp}$) of the active deposited on each component of the impactor, commencing with the induction port and proceeding in order through the impactor, using the following expression:

$$F_{m,comp} = \frac{m_{comp}}{M}$$

where, m_{comp} = mass associated with the components under evaluation;

M = total mass collected by the system.

Present $F_{m,comp}$ in order of location within the measurement equipment, beginning at the induction port and ending with the back-up filter of the impactor (Fig. 18). Where appropriate, $F_{m,comp}$ for adjacent stages of the impactor may be combined in order to report the mass fraction collected on a group of stages as a single value.

Determine the cumulative mass-weighed particle-size distribution of the aerosol size-fractionated by the impactor in accordance with the procedure given in inhalation preparation. Starting at the filter, derive a cumulative mass versus effective cut-off diameter of the respective stages (see Table 12 for the appropriate cut-off diameters at 15 litres per minutes). Plot the cumulative fraction of active substance versus cut-off diameter in a suitable format, for example logarithmic or log-probability format. Where appropriate, determine by interpolation the fraction either below a given size or between an upper and a lower size limit.

Table 12 - Cut-off sizes for Apparatus D at 15 litre per minutes

Stage	cut-off diameter (μm)
1	14.1
2	8.61
3	5.39
4	3.30
5	2.08
6	1.36
7	0.98

If necessary, and where appropriate, determine values for the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD), as appropriate.

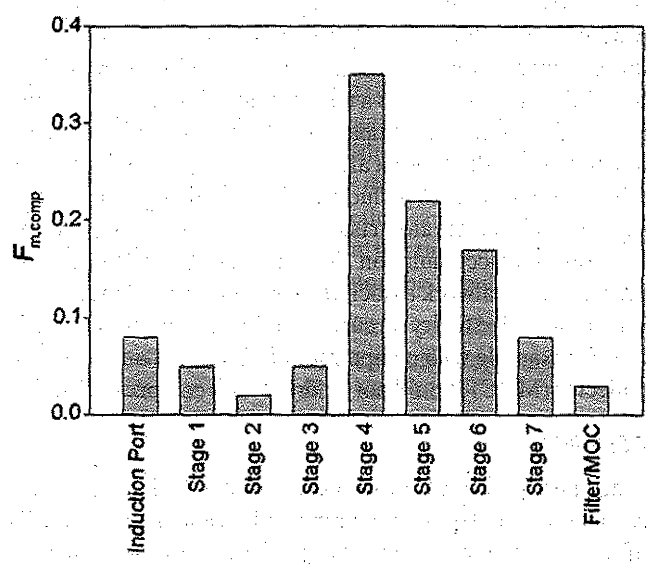


Fig. 18: Example of mass fraction of droplets presented in terms of location within the sampling system

Content of active ingredient on actuation of the valve test

The following test conditions are for use in preparations for inhalation. Specifically the methodology should be applied to pressurised inhalation products.

Content of active ingredient delivered by actuation of the valve

Remove the pressurised container from the actuator and remove all labels and markings which may be present on the container with a suitable solvent. Dry the container, replace in its actuator, shake for 30 seconds and prime the metering valve as follows. Discharge once to waste, wait for no less than 5 seconds and discharge again to waste. Remove the pressurised container from its actuator; clean the valve stem (internally and externally) and the valve ferrule by washing with a suitable solvent. Dry the complete valve assembly using an air line fitted with an appropriate narrow jet to ensure that all solvent is removed from the inside of the valve stem.

Place a stainless steel base plate that has three legs and a central circular indentation with a hole about 1.5 mm in diameter in a small vessel suitable for shaking and add the volume of solvent specified in the monograph. The size of the vessel is such that when the pressurised inhalation is discharged into the specified volume of solvent as described below the discharge takes place not less than 25 mm below the surface of the solvent.

Shake the pressurised container for about 30 seconds and place it inverted in the vessel. Discharge 10 deliveries below the surface of the solvent actuating the valve at intervals of not less than 5 seconds, maintaining the pressurised container in the vertical plane and discharging the pressurised inhalation through the hole in the centre of the base plate. (It may be necessary because of the nature of the formulation to shake the pressurised container between each actuation of the valve; where this is the case shaking should be carried out without removing the pressurised container from its inverted position in the vessel). Remove the pressurised container, wash it with the specified solvent and dilute the combined solution and washing to the volume specified in the monograph. Determine the amount of active ingredient by the method described under the assay and calculate the amount delivered from each actuation of the valve. The result lies within the range for the content of active ingredient stated in the monograph.

Insulin Preparations

Introduction

Insulin preparations are sterile preparations of human Insulin, bovine insulin, porcine insulin, Insulin lispro, Insulin lispro injection or Biphasic insulin lispro injection intended for subcutaneous injection into the human or animal body. They are either solutions or suspensions or they are prepared by combining solutions and suspensions. They contain not less than 90.0 per cent and not more than the equivalent of 110.0 per cent of the amount of insulin stated on the label.

Production

Insulin preparations are made by methods that are designed to ensure their sterility, to avoid the introduction of foreign contaminants, bacterial endotoxins and the growth of micro-organisms. The methods used should confer suitable properties with respect to the onset and duration of therapeutic action.

The use of excipients in the injections may be necessary, for example to make the preparation isotonic with respect to blood, to adjust the pH to the appropriate value, to prevent deterioration of the active substances or to provide adequate antimicrobial properties. Where appropriate, suitable substances may be added and suitable procedures carried out to confer the appropriate physical form on the insulin-containing component or components. Irrespective of the purpose for which additives are used, they should not to adversely affect the intended therapeutic action of the preparation or, at the concentration used, cause toxicity or undue local irritation.

In the course of production the strength of the insulin-containing component or components should be determined, where necessary, by adjustment so that the final preparation contains the required number of Units of insulin per ml.

Initial sterilisation of the insulin-containing component or components is done by filtration and subsequent procedures are carried out aseptically using materials that have been sterilised by suitable methods.

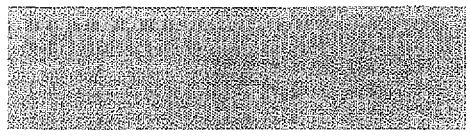
The final preparation is distributed aseptically into sterile glass or plastic containers or pre-filled syringes that are closed so as to exclude microbial contamination.

Tests

Insulin in the supernatant — *For preparations that are suspensions*

Not more than 2.5 per cent of the total insulin content, unless otherwise stated, determined in the following manner.

Centrifuge 10 ml of the suspension for 10 minutes and carefully separate the supernatant liquid from the residue. Determine the insulin content of the supernatant liquid (2.3.46) and calculate as a percentage of the total insulin content determined as described under Assay in the individual monograph.



Impurities with molecular masses greater than that of insulin

Determine by size-exclusion chromatography (2.4.16).

Test solution. Add 4 µl of *6 M hydrochloric acid* per millilitre of the preparation under examination, whether a suspension or a solution, to obtain a clear acid insulin solution. When sampling a suspension, agitate the material prior to sampling in order to obtain a homogeneous sample. If a suspension does not turn clear within 5 minutes of the initial addition of hydrochloric acid, add small aliquots of acid (less than 4 µl per millilitre) until a solution is obtained. Preparations with concentrations higher than 100 Units per ml need to be diluted with *0.01M hydrochloric acid* to avoid overloading the column with insulin monomer.

Resolution solution. Use a solution of insulin (approximately 4 mg per ml), containing more than 0.4 per cent of high molecular mass proteins. An injectable insulin preparation, whether a solution or a suspension, that has been clarified with a sufficient amount of *6 M hydrochloric acid*, containing the indicated percentage of high molecular mass proteins, or a solution prepared from insulin, dissolved in *0.01 M hydrochloric acid*, may be used. Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about ten days.

Maintain the solutions at 2° to 10° and use within 30 hours (soluble insulin injection) or 7 days (other insulin preparations). If an automatic injector is used, maintain the temperature at 2° to 10°.

Chromatographic system

- a stainless steel column 30 cm x 7.5 mm packed with *hydrophilic silica gel* (5 µm to 10 µm), of a grade suitable for the separation of insulin monomer from dimers and polymers,
- mobile phase: a filtered and degassed mixture of 15 volumes of *glacial acetic acid*, 20 volumes of *acetonitrile* and 65 volumes of a 1.0 g/l solution of *arginine*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume: 100 µl.

Before using a new column for chromatographic analysis, equilibrate by repeated injections of an insulin solution containing high molecular mass proteins. This can be done by at least three injections of the resolution solution. The column is equilibrated when repeatable results are obtained from two subsequent injections. If protamine-containing samples are to be analysed, the equilibration of the column is performed using a solution containing protamine.

Inject the resolution solution. When the chromatograms are recorded under the prescribed conditions, the retention times are: polymeric insulin complexes or covalent insulin-protamine complex, about 13 to 17 minutes, covalent insulin dimmer, about 17.5 minutes, insulin monomer, about 20 minutes, salts, about 22 min. If the sample solution contains preservatives, for example methyl paraben, *m*-cresol or phenol, these compounds elute later. The test is not valid unless the resolution, defined by the ratio of the height of the dimer peak to the height above the baseline of the valley separating the monomer and dimer peaks, is at least 2.0.

Inject the test solution. Record the chromatogram for approximately 35 min. In the chromatogram obtained, the sum of the areas of any peak with a retention time less than that of the insulin peak is not greater than 3.0 per cent (protamine-containing preparations) or 2.0 per cent (non-protamine containing preparations) of the total area of the peaks. Ignore any peak with a retention time greater than that of the insulin peak.

Related proteins

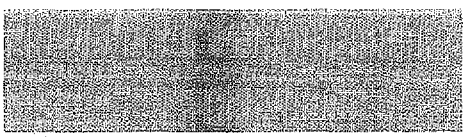
Determine by liquid chromatography (2.4.14) as described under Assay of Insulins (2.3.46), following the elution conditions as described in the Table:

Table

Time (min)	Mobile phase (a) (per cent v/v)	Mobile phase (b) (per cent v/v)	Comment
0-30	42	58	isocratic
30-44	42→11	58→89	linear gradient
44-50	11	89	isocratic

Maintain the solutions at 2° to 10° and use within 24 hours. Perform a system suitability check (resolution, linearity) as described under Assay of Insulins (2.3.46). If necessary, the relative proportions of the mobile phases may be adjusted to ensure complete elution of A21 desamido porcine insulin before commencement of the gradient. The profile of the gradient may also be adjusted to ensure complete elution of all insulin related impurities.

Inject 20 µl of the test solution and 20 µl of either reference solution (a), for insulin preparations containing 100 IU/ml, or reference solution (b), for insulin preparations containing 40 IU/ml. If necessary, adjust the injection volume to a volume between 10 µl and 20 µl in accordance with the results obtained in the test for linearity as described under Assay. Record the chromatograms for approximately 50 min. If necessary, make further adjustments to the mobile phase in order to ensure that the antimicrobial preservatives present in the test solution are well separated from the insulin and show a shorter retention time. A small reduction in the concentration of acetonitrile increases the retention time of the insulin peaks relatively



more than those of the preservatives. In the chromatogram obtained with either reference solution (a), or reference solution (b), as appropriate, A21 desamido insulin appears as a small peak after the principal peak and has a retention time of about 1.3 relative to the principal peak, due to insulin. In the chromatogram obtained with the test solution the area of the peak due to A21 desamido insulin is not greater than 5.0 per cent of the total area of the peaks; the sum of the areas of any other peaks, apart from those due to insulin and A21 desamido insulin is not greater than 6.0 per cent of the total area of the peaks. Disregard the peaks due to the preservatives and protamine (early eluting peaks).

Total zinc. Not more than the amount stated in the individual monograph, determined by either of the following methods.

A. To an accurately measured volume of the gently shaken injection containing 200 Units add 10 ml of *alkaline borate buffer pH 9.0*, 0.3 ml of *zincon solution* and sufficient *water* to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm, using as the blank a solution prepared by treating 5 ml of *water* instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of *zinc sulphate solution* and 6 volumes of *water*.

B. Determine by atomic absorption spectrometry (2.4.2).

Test solution. Shake the preparation gently and dilute a volume containing 200 Units of insulin to 25.0 ml with *0.01 M hydrochloric acid*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per millilitre) with *0.01 M hydrochloric acid*.

Reference solutions. Use solutions containing 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per millilitre, freshly prepared by diluting *zinc solution AAS (5 mg/ml Zn)* with *0.01 M hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Bacterial endotoxins (2.2.3). Less than 80 Endotoxin Units per 100 Units of insulin.

Sterility. Comply with the test for sterility (2.2.11).

Using following modifications in Biphasic Isophane Insulin Injection and Isophane Insulin Injection.

Add freshly prepared 1 per cent w/v solution of *ascorbic acid* in *Fluid A* given in sterility (2.2.11) to get clear solution of suspension.

Assay. Determine as described under Assay of Insulins ((2.3.46).

Storage. Unless otherwise prescribed, store in sterile, airtight, tamper-proof containers, protected from light, at a temperature of 2° to 8°. Insulin preparations should not to be frozen.

Labelling. The label states (a) the potency in Units per millilitre; (2) the concentration in terms of the number of milligrams of insulin per ml (for preparations containing both bovine insulin and porcine insulin the concentration is stated as the combined amount of both insulins); (3) where applicable, that the substance is produced by enzymatic modification of porcine insulin; (4) where applicable, that the substance is produced by recombinant DNA technology; (5) where applicable, the animal species of origin; (6) the preparation must not be frozen; (7) where applicable, that the preparation must be re-suspended before use.

Liposomal Preparations

Liposomal Injectable Preparations

Introduction

Liposomal Preparations are sterile dispersions for injections or infusions made up of phospholipids with or without cholesterol dispersed in aqueous vehicle. It may contain antioxidants, stabilizers and buffers. They are translucent to opalescent in appearance and may contain the active compound encapsulated in the vesicle or intercalated between the lipid bilayer. Their method of preparation may involve formation of the lipid film for hydration, hydration with agitation, and sizing of vesicles using different techniques like sonication, homogenization or extrusion.

Liposomal Preparations should not show any evidence of separation and show uniform appearance after shaking.

Tests

Particulate matter. Complies with the test stated under Parenteral Preparations (Injections).

Uniformity of content. Complies with the test stated under Parenteral Preparations (Injections).

Extractable volume. Complies with the test stated under Parenteral Preparations (Injections).

Sterility. Complies with the test stated under Parenteral Preparations (Injections).

Pyrogens. Complies with the test stated under Parenteral Preparations (Injections).

Vesicle size. Complies with the requirement of the test stated under individual monograph. Determine by Dynamic light scattering or Photon correlation spectroscopy or Laser diffraction.

Lamellarity. Lamellarity of the Liposomal Preparations should be defined. Lamellarity is determined by Freeze fracture microscopy or Transmission electron microscopy.

Powders for Liposomal Injection

Definition

Powders for Liposomal Injection are solid, sterile substances distributed in their final containers and which, when shaken with the prescribed volume of a prescribed sterile liquid rapidly form translucent to opalescent dispersion and practically particle-free uniform dispersions.

Freeze-dried Liposomal Products for parenteral use are considered as Powders for Liposomal Injection or infusion.

NOTE—After reconstitution of Powders for Liposomal injection, the reconstituted dispersion should comply with the monograph for Liposomal Preparations.

Labelling. The label states the instructions for the preparation of Liposomal Injections and Infusions.

Lotions

Lotions are liquids for cutaneous application that is intended to be applied to the unbroken skin without friction.

Tests

Uniformity of weight or volume. Unless otherwise specified, Lotions comply with the test for contents of packaged dosage forms (2.5.6).

Storage. Store at a temperature not exceeding 30°. Do not freeze.

Labelling. The label states that the lotion should be shaken before use.

Nasal Preparations

Nasal Preparations are liquid, semi-solid or solid preparations containing one or more medicaments and are intended for administration to the nostrils for local or systemic effects. They should as far as possible be non-irritating and should not affect the functions of the nasal mucosa and its cilia. They are supplied in single dose or multiple dose containers of glass VD or plastic with, if necessary, a suitable device for administration. They may also be supplied in pressurised containers with a suitable adaptor and with or without a metering dose valve.

Aqueous nasal preparations are usually isotonic and, when supplied in multiple dose containers, contain a suitable antimicrobial preservative except when the product itself has adequate antimicrobial properties.

During manufacture, packaging, storage and distribution of nasal preparations, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Tests

Uniformity of content. Comply with the test described under Parenteral Preparations.

Uniformity of weight. Nasal Preparations supplied in single dose containers comply with the test for contents of packaged dosage forms (2.5.6).

Nasal Drops, Solutions and Sprays

These are solutions, emulsions or suspensions intended for instillation or spraying into the nostrils. Emulsions should have a uniform appearance after shaking and should not show evidence of phase separation. Suspensions should be readily redispersible on shaking to give a smooth and stable suspension. In suspensions, the size of the dispersed particles should be such as to localise their deposition in the nostril.

Nasal Powders

These are powders intended for insufflation into the nostrils by means of a suitable device. The size of the particles should be such as to localise their deposition in the nostril.

Storage. Store protected from light and moisture.

Tests

Uniformity of content. Comply with the test described under Parenteral Preparations.

Uniformity of weight. Nasal Preparations supplied in single application containers comply with the test for contents of packaged dosage forms (2.5.6).

Ointments

Ointments are homogeneous, semi-solid preparations intended for external application to the skin or certain mucous membranes for emollient, protective, therapeutic or prophylactic purposes where a degree of occlusion is desired. They usually consist of solutions or dispersions of one or more medicaments in suitable bases. They are formulated using hydrophobic, hydrophillic or water-emulsifying bases to provide preparations that are immiscible, miscible or

emulsifiable with the skin secretion, respectively. The base should not produce irritation or sensitisation of the skin, nor should it retard wound healing; it should be smooth, inert, odourless or almost odourless, physically and chemically stable and compatible with the skin and with incorporated medicaments. The proportions of the base ingredients should be such that the ointment is not too soft or too hard for convenient use. The consistency should be such that the ointment spreads and softens when stress is applied.

Ointments may contain suitable auxiliary substances such as antioxidants, stabilisers, thickeners and emulsifiers and, when the base might support the growth of microbial contaminants, suitable antimicrobial preservatives.

During manufacture, packaging, storage and distribution of ointments, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

If an ointment is specifically intended for use on large wounds or on severely injured skin it should be sterile.

Ointments should not normally be diluted; if dilution is necessary care should be taken to choose the right diluent to avoid risk of instability or incompatibility.

Tests

Uniformity of weight. Comply with the test for contents of packaged dosage forms (2.5.6).

Sterility. When the ointment is labelled as sterile, it complies with the test for sterility (2.2.11).

Storage. Store at a temperature not exceeding 30° unless otherwise directed. Do not freeze.

Labelling. The label states (1) that the ointment is sterile, where necessary; (2) the name and concentration of any added antimicrobial preservative; (3) the storage conditions.

Oral Liquids

Oral Liquids are homogeneous liquid preparations, usually consisting of a solution, an emulsion or a suspension of one or more medicaments in a suitable vehicle*. They are intended for oral administration either undiluted or after dilution. They may contain auxiliary substances such as suitable dispersing, emulsifying, suspending, wetting, solubilising, thickening, stabilising agents and antimicrobial preservatives. They may also contain suitable sweetening, flavouring and permitted colouring agents. if saccharin, including its sodium and potassium salts, is used as a sweetening agent, its concentration in preparations meant for paediatric use should be restricted so as to limit its intake to 5 mg per kg of body weight.

Oral Liquids other than Oral Emulsions may be supplied as liquids or prepared just before use by dissolving or dispersing granules or powder in the liquid stated on the label. The granules or powder comply with the requirements stated under Oral Powders.

During manufacture, packaging, storage and distribution of oral liquids, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Oral Liquids should not be diluted and stored; where, however, the individual monograph directs dilution, the diluted Oral Liquid should be freshly prepared irrespective of the nature of the diluent. Diluted Oral Liquids may be less stable physically and chemically than the corresponding undiluted preparation and should be used within the period stated on the label.

Oral Liquids are variously known as Elixirs, Linctuses Mixtures, Oral Drops, Oral Emulsions, Oral Solutions, Oral Suspensions and Syrups. These terms are defined below.

Elixirs. Elixirs are clear, flavoured Oral Liquids containing one or more active ingredients dissolved in a vehicle that usually contains a high proportion of Sucrose or a suitable polyhydric alcohol or alcohols and may also contain Ethanol (95 per cent) or a dilute Ethanol.

Linctuses. Linctuses are viscous Oral Liquids containing one or more active ingredients dissolved in a vehicle that usually contains a high proportion of sucrose, other sugars or a suitable polyhydric alcohol or alcohols. Linctuses are intended for use in the treatment or relief of cough, and are sipped and swallowed slowly without the addition of water.

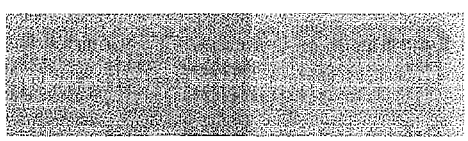
Mixtures. Mixtures are Oral Liquids containing one or more active ingredients dissolved, suspended or dispersed in a suitable vehicle. Suspended solids may separate slowly on keeping but are easily redispersed on shaking.

Oral Drops. Oral Drops are Oral Liquids that are intended to be administered in small volumes with the aid of a suitable measuring device such as a dropper.

Oral Emulsions. Oral Emulsions are Oral Liquids containing one or more active ingredients and are stabilised oil-in-water dispersions, either or both phases of which may contain dissolved solids. Solids may also be suspended in Oral Emulsions. Emulsions may exhibit phase separation but are easily reformed on shaking. The preparation remains sufficiently stable to permit a homogeneous dose to be withdrawn.

Oral Solutions. Oral Solutions are Oral Liquids containing one or more active ingredients dissolved in a suitable vehicle.

Oral Suspensions. Oral Suspensions are Oral Liquids containing one or more active ingredients suspended in a



suitable vehicle. Suspended solids may slowly separate on keeping but are easily redispersed.

In the manufacture of oral suspensions containing dispersed particles, measures shall be taken to ensure a suitable and controlled particle size with regard to the intended use of the product.

Syrups. Syrups are viscous Oral Liquids that may contain one or more active ingredients in solution. The vehicle usually contains large amounts of Sucrose or other sugars to which certain polyhydric alcohols may be added to inhibit crystallisation or to modify solubilisation, taste and other vehicle properties. Sugarless syrups may contain sweetening agents and thickening agents. Syrups may contain Ethanol (95 per cent) as a preservative or as a solvent to incorporate flavouring agents. Antimicrobial agents may also be added to Syrups.

Containers. Oral Liquids may be supplied in multiple dose or single dose containers. Oral Emulsions and Oral Suspensions should be packed in bottles sufficiently wide-mouthed to facilitate the flow of the contents. They are administered either in volumes such as 5 ml, or multiples of 5 ml, or in small volumes (drops). Each dose of a multiple dose Oral Liquid is administered by means of a suitable measuring device which is usually provided with the container.

Tests

Uniformity of content. Unless otherwise specified, single dose liquids in suspension form or powders or granules presented in single dose containers and that contain less than 10 mg or less than 10 per cent of active ingredient comply with the following test. For Oral Liquids containing more than one active ingredient, carry out the test for each active ingredient that corresponds to the above conditions. Empty each container as completely as possible and carry out the test on the individual contents of active ingredients.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the preparation has been shown to be within the accepted limits of the stated content.

Determine the content of active ingredient(s) of each of 10 containers taken at random using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision. The preparation complies with the test if the individual values thus obtained are all between 85 to 115 per cent of the average value. The preparation fails to comply with the test if more than one individual value is outside the limits 85 to 115 per cent of the average value or if any one individual value is outside the limits 75 to 125 per cent of the average value. If one individual value is outside the limits 85 to 115 per cent but within the limits 75 to 125 per cent of the average value, repeat the determination using another 20 containers taken at random. The preparation complies with the test if in the total sample of 30 containers not more than 3 individual values are outside the limits 85 to 115 per cent and not more than one is outside the limits 75 to 125 per cent of the average value.

Uniformity of weight/volume. Unless otherwise specified, Oral Liquids comply with the test for contents of packaged dosage forms (2.5.6).

Storage. Store Oral Liquids or powders and granules for the preparation of Oral Liquids in well-closed containers at temperatures not exceeding 30°.

Labelling. For Oral Liquids that are supplied as drops, the label states the number of drops per g of preparation if the dose is stated in drops or the number of drops per ml of preparation if the dose is stated in volume. For oral liquids supplied as granules or powder to be constituted before use, the label states (1) that the contents are meant for preparation of an Oral Liquid; (2) the directions for preparing the Oral liquid including the nature and quantity of the liquid to be used; (3) the conditions under which the constituted solution should be stored; (4) the period during which the constituted Oral Liquid may be expected to remain satisfactory for use when prepared and stored in accordance with the manufacturer's recommendations; (5) the strength in terms of the active ingredient(s) in a suitable dose-volume of the constituted preparation.

* The term vehicle means a carrier, composed of one or more excipients, for the active pharmaceutical ingredient(s) in a liquid preparation.

Oral Powders

Oral Powders are finely divided powders that contain one or more medicaments with or without auxilliary substances including, where specified, flavouring and colouring agents. However, addition of saccharin or its salts is not permitted in the preparations meant for paediatric use. They are intended to be taken internally with or without the aid of water or any other suitable liquid.

Oral Powders may be single dose or multiple dose preparations. For single dose powders, each dose is enclosed in a separate container, e.g., a sachet, a paper packet or a vial. With multiple dose powders it may be necessary to provide a measuring device capable of delivering the quantity prescribed.

Effervescent Oral Powders are intended to be dissolved or dispersed in water before administration.

In the manufacture of oral powders, means are taken to ensure a suitable particle size with regard to the intended use of the product. During manufacture, packaging, storage and distribution of oral powders, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Storage. Store Oral Powders in containers protected from moisture.

Tests

Uniformity of content. Unless otherwise specified, Oral Powders presented in single dose containers that contain less than 10 mg of active ingredient per dose or that contain less than 10 per cent w/w of active ingredient comply with the following test. For Oral Powders containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions. Empty each container as completely as possible and carry out the test on the individual contents of active ingredients.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the preparation has been shown to be within the accepted limits of the stated content.

Determine the content of active ingredient(s) of each of 10 containers taken at random using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision. The preparation complies with the test if the individual values thus obtained are all between 85 to 115 per cent of the average value. The preparation fails to comply with the test if more than one individual value is outside the limits 85 to 115 per cent of the average value or if any one individual value is outside the limits 75 to 125 per cent of the average value. If one individual value is outside the limits 85 to 115 per cent but within the limits 75 to 125 per cent of the average value, repeat the determination using another 20 containers taken at random. The preparation complies with the test if in the total sample of 30 containers not more than 3 individual values are outside the limits 85 to 115 per cent and not more than one is outside the limits 75 to 125 per cent of the average value.

NOTE — The test for Uniformity of content is not applicable to preparations containing multivitamins and trace elements.

Uniformity of weight. Unless otherwise specified, Oral Powders presented in single dose containers comply with the test for Uniformity of Weight of Single-Dose Preparations (2.5.3) and Oral Powders presented in other than single dose containers comply with the test for contents of packaged dosage forms (2.5.6).

Parenteral Preparations

Injectable Preparations

NOTE — The provisions of this monograph do not necessarily apply to Blood Products or Immunological Products because of their special nature and licensing requirements.

Introduction

Parenteral Preparations are sterile products intended for administration by injection, infusion or implantation into the body. They may be preparations intended for direct parenteral administration or they may be parenteral products for constituting or diluting prior to administration. There are five main types of Parenteral Preparations, namely, Injections, Infusions, Powders for Injection, Concentrated Solutions for Injection and Implants.

Production

Parenteral Preparations should be prepared by methods designed to ensure their sterility and to avoid the introduction of foreign contaminants, the presence of pyrogens or of bacterial endotoxins and the growth of micro-organisms.

Parenteral Preparations which are solutions or suspensions require vehicles in which the medicaments are incorporated. The most commonly used vehicle is Water for Injections that complies with the requirements for water for injections in bulk stated in the monograph on Water for injections. Any other suitable vehicles may be used provided they are safe in the volume of injections administered and also do not interfere with the therapeutic efficacy of the preparation or with its response to the prescribed tests and assays of the Pharmacopoeia. It may be necessary to include auxiliary substances to increase the stability or usefulness of the preparation, unless otherwise specified in the individual monograph. Such substances at the concentration at which they are used should not adversely affect the intended medicinal action of the preparation nor cause toxicity or local irritation and should not interfere with the responses to the specified tests and assays. No colouring agent may be added solely for the purpose of colouring the finished preparation.

Aqueous Parenteral Preparations for administration by the subcutaneous, intradermal, intramuscular, or in the case of large volumes, intravenous route, should if possible be made isotonic with blood by the addition of Sodium Chloride or other suitable substances. Buffering agents should not be used in preparations intended for intraocular or intracardiac injection, or in products that may gain access to the cerebrospinal fluid.

Parenteral Preparations that are packaged in multiple dose containers, regardless of the method of sterilisation employed, may contain suitable antimicrobial preservatives in appropriate concentration, unless otherwise directed in the individual monograph, or unless the active ingredients themselves are bacteriostatic. The effectiveness of the chosen preservative shall have been demonstrated during the development of a parenteral preparation.

Precautions to be taken for administration and for storage between successive withdrawals from such multiple dose

preparations should be indicated. Preservatives should not be added when the volume to be injected as a single dose exceeds 15 ml, unless otherwise justified, or when the preparation is intended for administration by the intraocular, intracardiac or intracisternal routes (or other route giving access to the cerebrospinal fluid).

Where the active ingredient is susceptible to oxidative degradation a suitable antioxidant may be added and/or the air in the container may be evacuated or displaced by oxygen-free nitrogen or other suitable inert gas.

Sterilisation. Methods of sterilisation that may be used in the manufacture of Parenteral Preparations are described in Chapter 5.3.

Containers. Containers for Parenteral Preparations are made as far as possible from materials that (1) are sufficiently transparent to permit visual inspection of the contents, except for implants; (2) do not adversely affect the quality of the preparation under the ordinary conditions of handling, shipment, storage, sale and use; (3) do not permit diffusion into or across the walls of the container or yield foreign substances into the preparation. Parenteral Preparations may be supplied in glass ampoules, vials or bottles or in other containers such as plastic bottles or bags or in prefilled syringes the integrity of which is ensured by suitable means. Requirements concerning containers are given in Chapter 6.2.

Single dose containers are used for administration of the contents on one occasion only and are to be preferred for all parenteral preparations. They may be used for intrathecal, intracardiac, intracisternal or intravenous injectable preparations. They contain sufficient of the Parenteral Preparation to permit the withdrawal and administration of the nominal dose using normal technique. They must be used for all parenteral preparations administered at one time in volumes of 10 ml or more.

Multiple dose containers permit the withdrawal of successive portions of the contents without removal or destruction of the closure and without changing the strength, quality or purity of the remaining portion. They may be used for intramuscular, subcutaneous or intracutaneous administration, but no multiple dose container may contain a total volume of injection sufficient to permit the withdrawal of more than ten doses, unless otherwise stated in the individual monograph. The period of time between the withdrawal of the first and final dose should not be unduly prolonged.

A multiple dose container for a sterile solid permits the addition of a suitable vehicle and withdrawal of portions of the resulting preparation in such a manner that the sterility of the product is maintained.

Closures. Vials or bottles are fitted with suitable closures that ensure a good seal, prevent the access of micro-organisms and other contaminants and usually permit the withdrawal of a part or the whole of the contents of the container without removal of the closure. The plastic or rubber materials of which the closure is composed must be compatible with the preparation and be sufficiently firm and elastic to allow the passage of a needle with minimal shedding of particles and to ensure that the puncture is resealed when the needle is withdrawn. Requirements concerning closures are given in Chapter 6.3.

Before use, closures should be washed with a suitable detergent and rinsed with and boiled in several changes of Purified Water. Closures made from rubber and synthetic materials are liable to absorb the ingredients of the parenteral preparation with which they are used, e.g., the preservative. When an antimicrobial preservative is used the closure, when necessary, should be placed in a solution of that preservative in Purified Water containing at least twice the concentration to be used in the preparation; the quantity of solution used should be sufficient to cover the closures and should be at least 2 ml for each g of the material. The vessel should then be closed and heated at an appropriate combination of time and temperature. After heating, the closures should be kept in the sealed container until required for use.

When the parenteral preparation with which the closures are to be used contains other added substances that are liable to be absorbed by the closure, these should be added to the solution in which the closures are to be heated in amounts equal to at least twice the concentration to be used in the parenteral preparation. Closures intended for containers of oily preparations should be made of oil-resistant materials.

Inspection. Good Manufacturing Practices require that each final container of a Parenteral Preparation be subjected individually to a physical inspection whenever the nature of the container permits and that every container the contents of which show evidence of contamination with visible foreign material be rejected.

Labelling. Containers of Parenteral Preparations should be labelled in a manner that sufficient area of the container remains uncovered for its full length or circumference to permit inspection of the contents. The label of a Parenteral Preparation states (1) the name of the Parenteral Preparation; (2) the strength in terms of the amount of active ingredient in percentage or in a suitable dose-volume; (3) the name and proportion of or antimicrobial preservative added; (4) the conditions under which the preparation should be stored.

In the case of Parenteral Preparations like Powders for Injection and Concentrated Solutions for Injection wherein a diluent is intended to be added before use, the label also states (1) the composition of the recommended diluent; (2) the conditions under which the constituted preparation should be stored; (3) the period within which the constituted solution should be used if it has been stored under the recommended conditions

of storage after constitution. In the case of Powders for Injection, the label also states the amount of diluent to be used to attain a specific concentration of the active ingredient in the solution or suspension so obtained whereas in the case of Concentrated Solutions for Injection, the amount of diluent to be used to attain a specific concentration and the final volume of the solution or suspension so obtained.

Injections

Injections are sterile solutions, emulsions or suspensions. They are prepared by dissolving, emulsifying or suspending the active ingredient(s) and any added substances in Water for Injection or in a suitable non-aqueous vehicle, or in a mixture of the two if they are miscible.

Injections that are emulsions should not show any evidence of separation and show a uniform appearance after shaking. The diameter of the globules of the dispersed phase of emulsions intended for intravenous injection must be decided with regard to the use of the preparation. Injections that are suspensions may show a sediment which is readily dispersible on shaking. The suspension remains sufficiently stable to enable a homogenous dose to be withdrawn from the container.

Tests

Particulate matter. Injections that are solutions, when examined under suitable conditions of visibility, are clear and practically free from particles that can be observed on visual inspection by the unaided eye. Injections that are supplied in containers with a nominal content of 100 ml or more comply with the test for particulate contamination (2.5.9).

Uniformity of content. Unless otherwise stated in the individual monograph, suspensions for injection that are presented in single dose containers and that contain less than 10 mg or less than 10 per cent of active ingredient comply with the following test. For suspensions for injection containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the preparation has been shown to be within accepted limits of the stated content.

Determine the content of active ingredient(s) of each of 10 containers taken at random, using the method given in the monograph or by any other suitable analytical method of equivalent accuracy and precision. The preparation under examination complies with the test if the individual values thus obtained are all between 85 and 115 per cent of the average value. The preparation under examination fails to comply with the test if more than one individual value is outside the limits 85 to 115 per cent of the average value or if any one individual value is outside the limits 75 to 125 per cent of the average value. If one individual value is outside the limits 85 to 115 per cent but within the limits 75 to 125 per cent of the average value, repeat the determination using another 20 containers taken at random. The preparation under examination complies with the test if in the total sample of 30 containers not more than one individual value is outside the limits 85 to 115 per cent and none is outside the limits 75 to 125 per cent of the average value.

NOTE — The test for Uniformity of content is not applicable to suspensions for injection containing multivitamins and trace elements.

Extractable volume

Suspensions and emulsions are shaken before withdrawal of the contents and before the determination of the density. Oily and viscous preparations may be warmed according to the instructions on the label, if necessary, and thoroughly shaken immediately before removing the contents. The contents are then cooled to 20-25° before measuring the volume.

Single-dose containers. Select 1 container if the nominal volume is 10 ml or more, 3 containers if the nominal volume is more than 3 ml and less than 10 ml, or 5 containers if the nominal volume is 3 ml or less. Take up individually the total contents of each container selected into a dry syringe of a capacity not exceeding 3 times the volume to be measured, and fitted with a 21-gauge needle not less than 2.5 cm in length. Expel any air bubbles from the syringe and needle, then discharge the contents of the syringe without emptying the needle into a standardised dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40 per cent of its graduated volume. Alternatively, the volume of the contents in millilitres may be calculated as the mass in grams divided by the density. For containers with a nominal volume of 2 ml or less, the contents of a sufficient number of containers may be pooled to obtain the volume required for the measurement provided that a separate, dry syringe assembly is used for each container. The contents of containers holding 10 ml or more may be determined by opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is not less than the nominal volume in case of containers examined individually, or, in case of containers with a nominal volume of 2 ml or less, is not less than the sum of the nominal volumes of the containers taken collectively.

Multidose containers. Labelled to yield a specific number of doses of a stated volume, select one container and proceed as directed for single-dose containers using the same number of separate syringe assemblies as the number of doses specified. The volume is such that each syringe delivers not less than the stated dose.

Cartridges and prefilled syringes

Select one container if the nominal volume is 10 ml or more, three containers if the nominal volume is more than 3 ml and less than 10 ml or 5 containers if the nominal volume is 3 ml or less. If necessary, fit the containers with the accessories required for their use (needle, piston and syringe) and transfer the entire content of each container without emptying the needle in a dry tared beaker by slowly and constantly depressing the piston. Determine the volume in millilitres calculated as the mass in grams divided by density.

The volume measured for each of the container is not less than the nominal volume.

Parenteral infusions (Large volume)

Select one container. Transfer the contents into a dry standard measuring cylinder of such a capacity that the volume to be measured occupies at least 40 per cent of the nominal volume of the cylinder.

Measure the volume transferred.

The volume is not less than the nominal volume.

Sterility (2.2.11). Injections comply with the test for sterility.

Bacterial endotoxins-Pyrogens. A test for bacterial endotoxins (2.2.3) is carried out or where justified and authorized, the test for pyrogens (2.2.8).

Infusions

Infusions are sterile aqueous solutions or emulsions with water as the continuous phase. They are free from pyrogens or bacterial endotoxins, are usually made isotonic with blood and do not contain any added antimicrobial preservatives. Intravenous Infusions that are emulsions do not show any evidence of phase separation. The diameter of the globules of the dispersed phase of emulsions must be decided with regard to the use of the preparation.

Tests

Intravenous Infusions comply with the requirements of tests stated under individual monographs and with the following requirements.

Particulate contamination. Intravenous Infusions that are solutions, when examined under suitable conditions of visibility, are clear and practically free from particles that can be observed on visual inspection by the unaided eye. Intravenous Infusions that are solutions and are supplied in containers with a nominal content of 100 ml or more comply with the test for particulate contamination (2.5.9).

Sterility (2.2.11). Intravenous Infusions comply with the test for sterility.

Bacterial endotoxins-Pyrogens. They comply with a test for bacterial endotoxins (2.2.3) or where justified and authorized, with the test for pyrogens (2.2.8). For pyrogen, inject 10 ml per kg of body weight into each rabbit, unless otherwise justified and authorized.

Powders for injection

Powders for injection are sterile, solid substances (including freeze-dried materials) which are distributed in their final containers and which, when shaken with the prescribed volume of the appropriate sterile liquid, rapidly form clear and practically particle-free solutions or uniform suspensions.

Tests

Powders for injection comply with the requirements of tests stated under individual monographs and with the following requirements.

Uniformity of content. Unless otherwise stated in the individual monograph, Powders for injection that contain 10 mg or less than 10 mg or less than 10 per cent of active ingredient or that have a unit weight equal to or less than 40 mg comply with the test for Uniformity of content described under Injections. For Powders for injection containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions. The test is not applicable to Powders for injection containing multivitamins and trace elements.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the preparation has been shown to be within accepted limits of the stated content.

Uniformity of weight. For Powders for injection that are required to comply with the test for Uniformity of content of all active ingredients, the test for Uniformity of weight is not required.

Remove any adherent labels from a container and wash and dry the outside. Open the container and immediately weigh the container and its contents. Empty the container as completely as possible by gentle tapping, rinse if necessary with *water* and then with *ethanol (95 per cent)* and dry at 100° to 105° for 1 hour or, if the nature of the container precludes such treatment, dry at a lower temperature to constant weight. Allow to cool in a desiccator and weigh. The difference between the weights represents the weight of the contents. Repeat the procedure with a further 19 containers and determine the average weight. Not more than two of the individual weights deviate from the average weight by more than 10 per cent and none deviates by more than 20 per cent.

Clarity of solution. Constitute the injection as directed on the label. (Not applicable to suspensions).

a) The solid dissolves completely, leaving no visible residue as undissolved matter.

b) The constituted injection is not significantly less clear than an equal volume of the diluent or of *water for injections* contained in a similar container and examined in the same manner.

Particulate matter. Constitute the injection as directed on the label; the solution is essentially free from particles of foreign matter that can be seen on visual inspection.

Sterility (2.2.11). Powders for injection comply with the test for sterility.

Bacterial endotoxins-Pyrogens. They comply with the requirements prescribed for injections or for infusions, after dilution or suspension in a suitable volume of liquid.

Concentrated Solutions for injection

Concentrated Solutions for injection are sterile solutions that are intended to be administered by injection or by intravenous infusion only after dilution with a suitable liquid.

Tests

After dilution Concentrated Solutions for injection comply with the requirements of tests for Injections or Infusions as appropriate.

Bacterial endotoxins-Pyrogens. They comply with the requirements prescribed for injections or for infusions, after dilution to a suitable volume.

Implants

Implants are sterile solid preparations of size and shape suitable for implantation into body tissues so as to release the active ingredient over an extended period of time. They are normally presented individually in sterile containers.

Tests

Sterility (2.2.11). Implants comply with the test for sterility.

Pessaries

Pessaries are solid preparations containing one or more active ingredients and are suitable for vaginal insertion. They are normally intended for use as a single dose.

The active ingredients are dissolved or dispersed in a suitable base containing one or more auxiliary substances that may be dispersible, soluble or insoluble in water. The auxiliary substances may be similar to the ones used for Suppositories or Tablets; such substances must be innocuous and therapeutically inert in the quantities present.

During manufacture, packaging, storage and distribution of pessaries, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Compressed Pessaries. Compressed Pessaries, also known as Vaginal Tablets, have the general characteristics of Uncoated Tablets but are usually large and of greater weight.

Storage. Store in well-closed containers, protected from moisture and from being crushed.

Moulded Pessaries. Moulded Pessaries are manufactured by pouring the liquefied mass containing the medicament(s) and auxiliary substances into moulds of suitable volume and cooling in order to solidify the mass. Auxiliary substances normally used are mixtures of mono-, di- and triglycerides of saturated fatty acids, macrogols, theobroma oil and gelatinous mixtures consisting of Gelatin, Glycerin and Water.

Moulded Pessaries are smooth and are usually ovoid in shape but may also be of various other shapes and of various volumes. When examined microscopically, their surfaces and longitudinal sections are normally of uniform texture except where the pessary consists of many layers.

Storage. Store in ventilated containers.

Shell Pessaries. Shell Pessaries, also known as Vaginal Capsules, are similar to Soft Capsules, differing only in their shape and size. They are commonly ovoid in shape, smooth and have a uniform appearance.

Storage. Store in well-closed containers.

Tests

Uniformity of container contents. Comply with the test for contents of packaged dosage forms (2.5.6).

Uniformity of content. The test is applicable to Pessaries that contain less than 10 mg or less than 10 per cent of active ingredient. For Pessaries containing more than one active ingredient carry out the test for each active ingredient that corresponds to the above conditions.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the pessaries has been shown to be within accepted limits of the stated content.

Carry out the test for Uniformity of content described under Capsules.

Uniformity of weight. This test is not applicable to Pessaries that are required to comply with the test for Uniformity of content for all active ingredients.

Weigh individually 20 pessaries, taken at random, and determine the average weight. Not more than two of the individual weights deviate from the average weight by more than 5 per cent and none deviates by more than 10 per cent.

Disintegration. *This test is not necessarily applicable to Pessaries intended for modified release or for prolonged local action.*

Carry out the disintegration test (2.5.1). Disintegration occurs in not more than 30 minutes for Compressed Pessaries and Shell Pessaries and in not more than 60 minutes for Moulded Pessaries.

Suppositories

Suppositories are solid preparations each containing one or more active ingredients and are suitable for rectal administration. They are normally intended for use as a single dose for local action or systemic absorption of the active ingredients.

The active ingredients are ground and passed through a sieve, if necessary, and dissolved or dispersed in a suitable basis that may be soluble or dispersible in water or that may melt at body temperature.

Suppositories may contain suitable auxiliary substances such as adsorbents, diluents, lubricants, antimicrobial preservatives and colouring agents permitted under the Drugs and Cosmetics Rules, 1945.

Moulded Suppositories. Moulded Suppositories are manufactured by liquefying by heating the mass containing the medicament(s) and auxiliary substances and then pouring the mass into moulds of suitable volume and cooling in order to solidify the mass. In some cases, the solid medicated mass may be cold-moulded by compression in a suitable matrix.

Moulded Suppositories have the characteristics of Moulded Pessaries.

Shell Suppositories. Shell Suppositories, also known as Rectal Capsules, are generally similar to Soft Capsules except that they may have lubricating coatings.

Shell Suppositories have the characteristics of Shell Pessaries.

During manufacture, packaging, storage and distribution of suppositories, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Tests

Moulded Suppositories and Shell Suppositories comply with the tests stated under Moulded Pessaries and Shell Pessaries respectively.

Storage. Store in well-closed containers.

Tablets

NOTE — The provisions of this monograph do not necessarily apply to tablets intended for use other than by oral administration such as Vaginal preparations or Oromucosal preparations and to lozenges, oral pastes and oral gums.

Definition

Tablets are solid dosage forms each containing a unit dose of one or more medicaments. They are intended for oral administration. Some tablets are swallowed whole or after being chewed, some are dissolved or dispersed in water before administration and some are retained in the mouth where the active ingredient is liberated.

Tablets are usually solid, right circular cylindrical, the end surfaces of which are flat or convex and the edges of which may be bevelled. They may exist in other shapes like triangular, rectangular, etc also. They may have lines or break-marks and may bear a symbol or other markings. They are sufficiently hard to withstand handling without crumbling or breaking. Tablets may bear a break-mark or break-marks.

Because of their composition, method of manufacture or intended use, tablets present a variety of characteristics and consequently there are several categories of tablets.

Tablets may be coated. Where coating is essential, the monograph states 'The tablets are coated'. In all other cases, coating is optional. Unless otherwise directed, tablets may be coated in one of different ways.

Production

Tablets are obtained by compression of uniform volumes of powders or granules or beads or pellets by applying high pressure and using punches and dies. The particles to be compressed consist of one or more medicaments, with or without auxiliary substances such as diluents, binders, disintegrating agents, lubricants, glidants, permitted colours and substances capable of modifying the behaviour of the medicaments in the digestive tract. Such substances must be innocuous and therapeutically safe in the quantities present.

In the production of tablets, measures are taken to ensure that they have sufficient strength to avoid crumbling or breaking on handling or subsequent handling. Chewing tablets are manufactured to ensure that they are easily crushed by chewing.

Subdivision of tablets. Tablets may bear a break-mark and may be subdivided in parts either to ease the intake of the medicinal product or to comply with the posology. In order to ensure

that the patient will receive the intended dose, the efficacy of the break-mark(s) must be assessed during the development of the product, in respect of uniformity of mass of the subdivided parts. Each authorized dose must be tested using the following test.

Take 30 tablets at random, break them by hand and from all the parts obtained from 1 tablet, take 1 part for the test and reject the other part(s). Weigh each of the 30 parts individually and calculate the average mass. The tablets comply with the test if not more than 1 individual mass is outside the limits of 85 per cent to 115 per cent of the average mass. The tablets fail to comply with the test if more than 1 individual mass is outside these limits or if 1 individual mass is outside the limits of 75 per cent to 125 per cent of the average mass.

During manufacture, packaging, storage and distribution of tablets, suitable means shall be taken to ensure their microbial quality; acceptance criteria for microbial quality are given in Chapter 2.2.9.

Tests

NOTE —Unless otherwise stated below or in the individual monograph, the following tests apply to all categories of tablets.

Uniformity of container contents. Tablets comply with the test for contents of packaged dosage forms (2.5.6).

Content of active ingredients. Determine the amount of active ingredient(s) by the method described in the Assay and calculate the amount of active ingredient(s) per tablet. The result lies within the range for the content of active ingredient(s) stated in the monograph. This range is based on the requirement that 20 tablets, or such other number as may be indicated in the monograph, are used in the Assay. Where 20 tablets cannot be obtained, a smaller number, which must not be less than 5, may be used, but to allow for sampling errors the tolerances are widened in accordance with Table 1. The requirements of Table 1 apply when the stated limits are between 90 and 110 per cent. For limits other than 90 to 110 per cent, proportionately smaller or larger allowances should be made.

Table 1

Weight of active ingredients in each tablet	Subtract from lower limit for samples of			Add to the upper limit for samples of		
	15	10	5	15	10	5
0.12 g or less	0.2	0.7	1.6	0.3	0.8	1.8
More than 0.12 g but less than 0.3 g	0.2	0.5	1.2	0.3	0.6	1.5
0.3 g or more	0.1	0.2	0.8	0.2	0.4	1.0

Uniformity of content (2.5.4). This test is applicable to tablets that contain 10 mg or less than 10 mg or less than 10 per cent w/w of active ingredient. For tablets containing more than one active ingredient carry out the test for each active ingredient that corresponds to the aforementioned conditions.

Irrespective of their strengths the test is applicable to coated tablets other than film coated tablets.

The test for Uniformity of content should be carried out only after the content of active ingredient(s) in a pooled sample of the tablets has been shown to be within accepted limits of the stated content.

The test for Uniformity of content is not applicable to tablets containing multivitamins and trace elements."

Applicability of test for Uniformity of content for tablets

Table 2

Type of Tablets	For tablets containing 10 mg or less than 10 per cent w/w of active ingredient	For tablets containing more than 10 mg or more than 10 per cent w/w of active ingredient
Uncoated tablets	Applicable	Not Applicable
Film coated tablets	Applicable	Not Applicable
Other coated tablets	Applicable	Applicable*

*Unless otherwise justified and authorized.

Uniformity of weight (2.5.3).This test is not applicable to coated tablets other than film-coated tablets and to tablets that are required to comply with the test for uniformity of content for all active ingredients.

Dissolution (2.5.2). Where required, the requirements for this test are given in the individual monographs. Where a dissolution test is prescribed, the disintegration test may not be necessary.

Uncoated Tablets

Uncoated tablets may be single-layer tablets resulting from a single compression of particles or multi-layer tablets consisting of parallel layers obtained by successive compression of particles of different compositions. No treatment is applied to such tablets after compression. Any added substances are not specifically intended to modify the release of their active ingredient(s) in the digestive fluids.

The addition of flavouring agents to uncoated tablets other than multi-layer tablets is not official unless permitted in the individual monograph. Uncoated Tablets have the general

characteristics of tablets. When a broken section of an uncoated tablet is examined under a lens, either a relatively uniform texture (single-layer tablets) or a stratified structure (multi-layer tablets) is seen; there are no signs of coating.

Tests

Disintegration (2.5.1). Use *water* as the liquid. Add a disc to each tube. Operate the apparatus for 15 minutes, unless otherwise stated in the individual monograph. Examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, repeat the test on a further 6 tablets omitting the discs. The tablets comply with the test if all 6 tablets have disintegrated.

The test does not apply to chewable tablets.

Coated Tablets

Coated tablets are tablets covered with one or more layers of mixtures of various substances such as resins, gums, gelatin, inactive and insoluble fillers, sugars, plasticisers, polyhydric alcohols, waxes, colouring matter authorized by the competent authority and sometime flavouring substances and active substances, etc. The coating may also contain medicaments. In compression-coated tablets, the coating is applied by compressing around the tablets granules prepared from tablet excipients such as lactose, calcium phosphate, etc. Substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs. When the coating is thin, the tablets are described as film-coated.

Coated tablets may contain flavouring agents.

Coated tablets have a smooth, usually polished and often coloured, surface; a broken section examined under a lens shows a core surrounded by one or more continuous layers of a different texture.

Tests

Disintegration (2.5.1). *For coated tablets other than film-coated tablets.*

Use *water* as the liquid. Add a disc to each tube. Operate the apparatus for 60 minutes, unless otherwise stated in the individual monograph. Examine the state of the tablets. If any of the tablets has not disintegrated, repeat the test on a further 6 tablets, replacing *water* with *0.1 M hydrochloric acid*. The tablets comply with the test if all 6 tablets have disintegrated.

Film-coated Tablets

Carry out the test described above but operate the apparatus for 30 minutes, unless otherwise stated in the individual monograph.

If coated tablets fail to comply because of adherence to the discs, repeat the test on a further 6 tablets omitting the discs. The tablets comply with the test if all 6 tablets have disintegrated in the acid medium.

The test does not apply to chewable tablets.

Dispersible Tablets

Dispersible tablets are uncoated or film-coated tablets that produce a uniform dispersion in water and may contain permitted flavouring and sweetening agents. However, if saccharin, including its sodium and potassium salts, is used as a sweetening agent, its concentration in dispersible tablets meant for paediatric use should be restricted so as to limit its intake to 5 mg/kg of body weight.

Tests

Disintegration (2.5.1).Use *water* as the liquid. Determine at 24° to 26° and operate the apparatus for 3 minutes.

Uniformity of dispersion. Place 2 tablets in 100 ml of *water* and stir gently until completely dispersed. A smooth dispersion is obtained which passes through a sieve screen with a nominal mesh aperture of 710 µm (sieve number 22).

Effervescent Tablets

Effervescent tablets are uncoated tablets generally containing acidic substances and either carbonates or bicarbonates which react rapidly in the presence of water to release carbon dioxide and they may contain permitted flavouring agents. They are intended to be dissolved or dispersed in water before administration.

Tests

Disintegration (2.5.1). Place one tablet in a 250-ml beaker containing 200 ml of *water* at 20° to 30°; numerous gas bubbles are evolved. When the evolution of gas around the tablet or its fragments has ceased the tablet shall have disintegrated, being either dissolved or dispersed in the water so that no agglomerates of particles remain. Repeat the operation on a further 5 tablets. The tablets comply with the test if each of the 6 tablets disintegrates in the manner prescribed within 5 minutes, unless otherwise stated in the individual monograph.

Modified-release Tablets

Modified-release tablets are coated or uncoated tablets containing auxiliary substances or prepared by procedures that, separately or together, are designed to modify the rate or the place at which the active ingredient is released.

Modified-release tablets include gastro-resistant tablets and prolonged-release tablets.

Gastro-resistant Tablets

Gastro-resistant tablets are delayed-release tablets that are intended to resist the gastric fluid but to release their active ingredient(s) in the intestinal fluid. For this purpose substances such as cellulose acetate phthalate and anionic copolymers of methacrylic acid and its ethers are used for providing tablets with a gastric-resistant coating (enteric coating) or for covering either granules or particles with gastric-resistant coating.

These tablets may be labeled as gastro-resistant tablets or enteric coated tablets as the case may be.

Tablets covered with gastro resistant coating conform to the definition of Coated Tablets.

Tests

Disintegration (2.5.1). If the tablet has a soluble external coating, immerse the basket in *water* at room temperature for 5 minutes. Suspend the assembly in the beaker containing *0.1 M hydrochloric acid* and operate without the discs for 120 minutes, unless otherwise stated in the individual monograph. Remove the assembly from the liquid. No tablet shows signs of cracks that would allow the escape of the contents of disintegration, apart from fragments of coating. Replace the liquid in the beaker with *phosphate buffer pH 6.8*, add a disc to each tube and operate the apparatus for a further 60 minutes. Remove the assembly from the liquid. The tablets pass the test if all six have disintegrated.

Dissolution (2.5.2). For tablets prepared from granules or particles already covered with an enteric coating, the dissolution test is carried out to demonstrate the appropriate release of the active substance(s).

Prolonged-release Tablets

Prolonged-release tablets, also known as sustained-release tablets, controlled-release tablets or extended-release tablets are tablets formulated in such a manner as to make the contained active ingredient available over an extended period of time after ingestion based on therapeutic justification.

Tests

Dissolution (2.5.2). The test should be designed to demonstrate the appropriate release of the active substance(s). The manufacturer is expected to give specifications for drug release at 3 or more test-time points. The first point should be set after a testing period corresponding to a dissolved amount of typically 20 per cent to 30 per cent. The second point should define the dissolution pattern and should be set typically 45 per cent to 55 per cent release. The final point should ensure almost complete release that is generally understood as more than 80 per cent release.

NOTE — Above specifications are non mandatory.

Carry out the test as per the manufacturer's specification for the indicated test-times.

Soluble Tablets

Soluble tablets are uncoated tablets or film-coated tablets that are to be dissolved in water before use. The solution produced may be slightly opalescent due to added substances used in the manufacture of the tablets.

Tests

Disintegration (2.5.1). Soluble tablets disintegrate within 3 minutes. The test is carried out using *water* as liquid medium at 15°to 25°.

Tablets for Use in the Mouth

Tablets for use in the mouth are usually uncoated tablets formulated to disintegrate orally or be chewed or to effect a slow release and local action of the active ingredient (lozenges) or the release and absorption of the active ingredient under the tongue (sublingual tablets). Chewable tablets and lozenges may contain flavouring agents. These can be categorized as

Orodispersible Tablets (Mouth Dissolving Tablets)

Orodispersible tablets are uncoated tablets intended to be placed in the mouth where they disperse rapidly before being swallowed.

Tests

Disintegration (2.5.1). Orodispersible tablets disintegrate within 3 minutes, using water as liquid medium.

Sublingual Tablets

Sublingual tablets are intended to be placed below the tongue for administration.

Tests

Disintegration (2.5.1). Sublingual tablets disintegrate within 3 minutes, at 15°to 25°.

Chewable Tablets

Chewable tablets are intended to be chewed before being swallowed.

Chewable tablets are prepared to ensure that they are easily crushed by chewing.

For chewable tablets disintegration test does not apply unless otherwise stated in the monograph.

Oral lyophilisates. Oral lyophilisates are solid preparations intended either to be placed in the mouth or to be dispersed (or dissolved) in water before administration.

Oral lyophilisates are obtained by freeze-drying (lyophilisation), involving division into single doses, freezing, sublimation and drying of usually aqueous, liquid or semisolid preparations.

Tests

Disintegration (2.5.1). Place 1 oral lyophilisates in a beaker containing 200ml of water at 15°to 25°. It disintegrates within 3 minutes. Repeat the test on 5 other oral lyophilisates. They comply with the test if all 6 have disintegrated.

Water (2.3.43). Oral lyophilisates comply with the test, the limits are approved by the competent authority.

Labelling. The label states whether or not the tablets are coated.

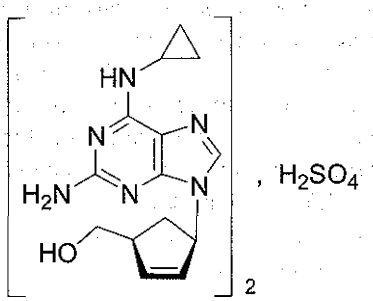
Where applicable the label states that the tablets should be chewed before swallowing.

The label states the common name of the colour used.

DRUG SUBSTANCES, DOSAGE FORMS AND PHARMACEUTICAL AIDS

A

Abacavir Sulphate



$(C_{14}H_{18}N_6O)_2,H_2SO_4$ Mol. Wt. 670.8

Abacavir Sulphate is {(1*S*,4*R*)-4-[2-amino-6-(cyclopropylamino)9*H*-purin-9-yl]cyclopent-2-enyl}methanol sulphate.

Abacavir Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{14}H_{18}N_6O)_2$, H_2SO_4, calculated on the anhydrous basis.

Category. Antiretroviral.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *abacavir sulphate IPRS* or with the reference spectrum of abacavir sulphate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of sulphates (2.3.1).

Tests

Specific optical rotation (2.4.22). −38.0° to −32.0°, determined in a 0.5 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay using the following solutions.

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.3 per cent.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *abacavir sulphate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* and 2 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, adjusted to pH 6.0 with *triethylamine*, 10 volumes of *methanol* and 15 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject the reference solution.The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $(C_{14}H_{18}N_6O)_2,H_2SO_4$.

Storage. Store at a temperature not exceeding 30°.

Abacavir Oral Solution

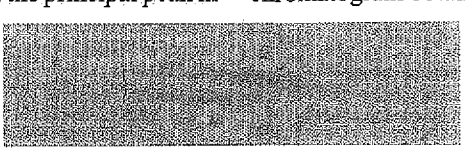
Abacavir Sulphate Oral Solution

Abacavir Oral Solution contains Abacavir Sulphate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of abacavir $C_{14}H_{18}N_6O$. It may contain one or more suitable buffers, colours, flavours, preservatives, stabilizers, sweeteners, and suspending agents.

Usual strength. 20 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.



Tests

pH (2.4.24). 4.6 to 5.0.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve a quantity of the oral solution containing 50 mg of abacavir in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve a quantity of *abacavir sulphate IPRS* in the mobile phase to obtain a solution containing 0.05 per cent w/v of abacavir.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* and 2 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, adjusted to pH 6.0 with *triethylamine* and 15 volumes of *acetonitrile,*
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve a quantity of the oral solution containing 60 mg of abacavir in the mobile phase and dilute to 100.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve a quantity of *abacavir sulphate IPRS* in the mobile phase to obtain a solution containing 0.06 per cent w/v of abacavir. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* and 2 g of *tetrabutyl ammonium hydrogen sulphate* in 1000 ml of *water,* adjusted to pH 6.0 with *triethylamine* and 15 volumes of *acetonitrile,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml of the oral solution (2.4.29) and calculate the content of $C_{14}H_{18}N_6O$ weight in volume.

Storage. Store at a temperature not exceeding 30°. Do not freeze.

Labelling. The label states the strength in terms of the equivalent amount of abacavir.

Abacavir Tablets

Abacavir Sulphate Tablets

Abacavir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of abacavir, $C_{14}H_{18}N_6O$.

Usual strength. 300 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 75 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter.
Determine by liquid chromatography (2.4.14).
Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *abacavir sulphate IPRS* in the dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* and 2 g of *tetrabutyl ammonium hydrogen sulphate* in 1000 ml of *water*, adjusted to pH 6.0 with *triethylamine* and 15 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 10 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{18}N_6O$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{14}H_{18}N_6O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Abacavir in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve a quantity of *abacavir sulphate IPRS* in the mobile phase to obtain a solution containing 0.05 per cent w/v of abacavir.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* and 2 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, adjusted to pH 6.0 with *triethylamine* and 15 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Abacavir in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve a quantity of *abacavir sulphate IPRS* in the mobile phase to obtain a solution containing 0.05 per cent w/v of abacavir. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* and 2 g of *tetrabutyl ammonium hydrogen sulphate* in 1000 ml of *water*, adjusted to pH 6.0 with *triethylamine* and 15 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency in not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{18}N_6O$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of abacavir.

Abacavir and Lamivudine Tablets

Abacavir Sulphate and Lamivudine Tablets

Abacavir and Lamivudine Tablets contain abacavir sulphate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of abacavir, $C_{14}H_{18}N_6O$ and lamivudine, $C_8H_{11}N_3O_3S$.

Usual strengths. 600 mg abacavir and 300 mg Lamivudine.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 75 mg of *abacavir sulphate IPRS* and 30 mg of *lamivudine IPRS* in 10 ml of *methanol* and dilute to 100.0 ml with the dissolution medium.

Use the chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{18}N_6O$ and $C_8H_{11}N_3O_3S$ in the medium.

Q. Not less than 80 per cent of the stated amounts of $C_{14}H_{18}N_6O$ and $C_8H_{11}N_3O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 95 volumes of mobile phase A and 5 volumes of mobile phase B.

Test solution. Disperse a quantity of the powdered tablets containing 100 mg of abacavir in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *lamivudine IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a buffer solution prepared by dissolving 1.9 g of *ammonium acetate* in 900 ml of *water*, adjusted to pH 3.8 with *glacial acetic acid* and dilute to 1000 ml with *water*,

  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
20	95	5
40	30	70
45	95	5
50	95	5

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 3.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 60 mg of Abacavir in 20 ml of *0.1 M hydrochloric acid* and dilute to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve 35 mg of *abacavir sulphate IPRS* and 15 mg of *lamivudine IPRS* in 15 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 7.66 g of *ammonium acetate* in 1000 ml of 0.5 per cent w/v solution of *glacial acetic acid* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the contents of $C_{14}H_{18}N_6O$ and $C_8H_{11}N_3O_3S$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Abacavir, Lamivudine and Zidovudine Tablets

Abacavir, Lamivudine and Zidovudine Tablets contain abacavir sulphate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of abacavir, $C_{14}H_{18}N_6O$ lamivudine, $C_8H_{11}N_3O_3S$ and zidovudine, $C_{10}H_{13}N_5O_4$.

Usual strength. 300 mg Abacavir, 150 mg Lamivudine and 300 mg Zidovudine.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution containing 0.035 per cent w/v of *abacavir sulphate IPRS*, 0.015 per cent w/v *lamivudine IPRS* and 0.03 per cent w/v of *zidovudine IPRS* in the dissolution medium.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (Such as Restek's Pinnacle II C-18),
- column temperature: 50°,
- mobile phase: a mixture of 88 volumes of a buffer solution prepared by dissolving 1 g of *octanesulphonic acid* and 1 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 12 volumes of *acetonitrile*,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the resolution between lamivudine and zidovudine peaks is not less than 2.5, the column efficiency for lamivudine, zidovudine and abacavir peaks is not less than 700, 1200 and 2000 theoretical plates respectively, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the reference solution and the test solution.

Calculate the contents of $C_{14}H_{18}N_6O$, $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$ in the medium.

Q. Not less than 70 per cent of the stated amounts of $C_{14}H_{18}N_6O$, $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A 0.2 per cent v/v solution of *orthophosphoric acid* in a mixture of 70 volumes of *water* and 30 volumes of *methanol*.

Test solution. Disperse a quantity of the powdered tablets containing 75 mg of Lamivudine in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.075 per cent w/v solution of *lamivudine IPRS* in the solvent mixture.

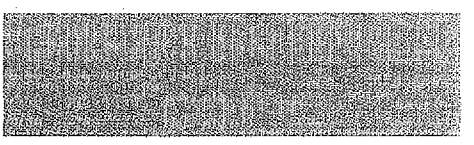
Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 70 volumes of *methanol*, 30 volumes of *acetonitrile* and 0.4 volume of *tetrahydrofuran*,

  B. a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
- a gradient programme using the conditions given below,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Flow rate (ml per min.)
0	2	98	1
10	2	98	1
25	20	80	1
28	20	80	1
50	30	70	1
60	35	65	1.3
63	35	65	1.3
66	2	98	1
80	2	98	1



Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 1.5 for each component.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *water* and 50 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 150 mg of Abacavir in 100 ml of *water*, add 80 ml of *methanol* and dilute to 200.0 ml with *methanol*. Dilute 10.0 ml of the solution to 25.0 ml with the solvent mixture.

Reference solution. A solution containing 0.35 per cent w/v of *abacavir sulphate IPRS*, 0.15 per cent w/v *lamivudine IPRS* and 0.30 per cent w/v of *zidovudine IPRS* in the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Kromasil C-18),
- column temperature: 50°,
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1 g of *octanesulphonic acid* and 1 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 4.5 with *orthophosphoric acid* and 35 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 10 µl.

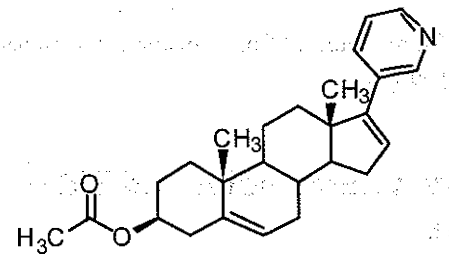
Inject the reference solution. The test is not valid unless the column efficiency for lamivudine is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the reference solution and the test solution.

Calculate the contents of $C_{14}H_{18}N_6O$, $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Abiraterone Acetate



$C_{26}H_{33}NO_2$ Mol. Wt. 391.6

Abiraterone Acetate is 17-(3-pyridinyl)androsta-5,16-dien-3β-yl acetate.

Abiraterone Acetate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{26}H_{33}NO_2$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *abiraterone acetate IPRS* or with the reference spectrum of abiraterone acetate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 60 mg of the substance under examination in *methanol* and dilute to 20.0 ml with *methanol*.

Reference solution. A 0.0015 per cent w/v solution of *abiraterone acetate IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with phenyl group (3.5 µm) (Such as Zorbax SB-Phenyl),
- mobile phase: A. a mixture of 70 volumes of *water* and 30 volumes of *acetonitrile*,
  B. a mixture of 90 volumes of *acetonitrile* and 10 volumes of *methanol*,
- a gradient programme using the conditions given below,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Flow (ml.per min.)
0	45	55	0.5
18	45	55	0.5
18.1	45	55	1.0
35	0	100	1.0
35.1	45	55	0.5
43	45	55	0.5

Name	Relative retention time	Correction factor
Abiraterone acetate impurity A[1]	0.22	1.3
Abiraterone acetate impurity B[2]	0.41	1.51
Abiraterone acetate impurity C[3]	0.49	0.49
Abiraterone acetate impurity D[4]	0.58	0.89
Abiraterone	0.67	0.44
Abiraterone acetate (Retention time: about 26 minute)	1.0	—
Reduced impurity[5]	1.07	1.3

[1](3β)- 3-hydroxy-androsta-5- ene-17-one,

[2](3β)- 3-acetoxy-androsta-5- ene-17-one,

[3]17-iodo-androsta-5,16-diene-3-beta-ol,

[4]5, 16-pregnadien-3B-acetoxy-20-one,

[5](3β)- 17-(pyridine-3-yl)androsta-16- ene-3-ol.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to abiraterone acetate impurities A, B, C, D and reduced impurity is not more than the area of principal peak in the chromatogram obtained with the reference solution (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm)

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30 mg of the substance under examination in 100.0 ml with *methanol.*

Reference solution. A 0.03 per cent w/v solution of *abiraterone acetate IPRS* in *methanol.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with phenyl group (3.5 µm) (Such as Zorbax SB-Phenyl),
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 40 volumes of *methanol* and 10 volumes of *water*,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theorotical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{33}NO_2$.

Storage. Store protected from moisture.

Abiraterone Acetate Tablets

Abiraterone Acetate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of abiraterone acetate, $C_{26}H_{33}NO_2$.

Usual strength. 250 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution. (2.5.2)

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.0565 M sodium dihydrogen orthophosphate monohydrate* in 0.25 per cent w/v solution of *sodium lauryl sulphate*, adjusted to pH 4.5 with *orthophosphoric acid,*

Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *abiraterone acetate IPRS* in dissolution medium and dilute quantitatively with the dissolution medium to obtain a solution of similar concentration as the test solution.

Use the chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{33}NO_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{26}H_{33}NO_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 150 mg of abiraterone acetate in 35 ml of *methanol* with the aid of ultrasound for 10 minutes and dilute to 50.0 ml with *methanol*, mix well and filter.

Reference solution. A 0.0006 per cent w/v solution of *abiraterone acetate IPRS* in the *methanol*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as Waters X bridge shield RP 18),
- mobile phase: A. *0.01M potassium dihydrogen orthophosphate,*
  B. a mixture of 90 volumes of *acetonitrile* and 10 volumes of *water;*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 252 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	45	55
25	30	70
40	22	78
45	22	78
46	45	55
53	45	55

Inject reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (1.0 per cent). Ignore any peak with an area less than 0.5 times of the principal peak in the chromatogram obtained with reference solution. (0.1 per cent).

Other tests. Complies with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 250 mg of Abiraterone Acetate in 150 ml of *methanol* with the aid of ultrasound for 15 minutes and dilute to 200.0 ml with *methanol*, mix well and filter. Dilute 5.0 ml to 20.0 ml with *methanol*.

Reference solution. A 0.031 per cent w/v solution of *abiraterone acetate IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as Zorbax SB-C18),
- mobile phase: a mixture of 10 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 90 volumes of a mixture of 90 ml of *acetonitrile* and 10 ml of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- injection volume: 5 µl.

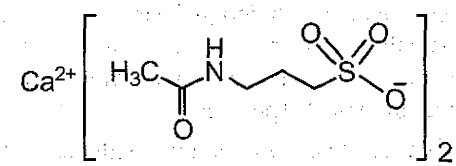
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{33}NO_2$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Acamprosate Calcium



$C_{10}H_{20}CaN_2O_8S_2$ Mol.Wt.400.5

Acamprosate Calcium is calcium bis(3-acetamidopropane-1-sulphonate).

Acamprosate Calcium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{10}H_{20}CaN_2O_8S_2$, calculated on the dried basis.

Category. Indicated for treatment of alcohol dependence.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acamprosate calcium IPRS* or with the reference spectrum of acamprosate calcium.

B. It gives reaction (A) of calcium (2.3.1).

Tests

Solution A. A 5.0 per cent w/v solution in *carbon dioxide-free water*.

Appearance of solution. Solution A is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 5.5 to 7.0 for solution A.

Impurity A (Homotaurine). Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.4 g of the substance under examination in *water* and dilute to 20.0 ml with *water*. Dilute 10.0 ml of the solution to 100.0 ml with *borate buffer solution pH 10.4*. Transfer 3.0 ml of the solution in a 25 ml ground-glass-stoppered tube. Add 0.15 ml of a freshly prepared 0.5 per cent w/v solution of *fluorescamine* in *acetonitrile*. Shake immediately and vigorously for 30 seconds. Place in a water-bath at 50° for 30 minutes. Cool under a stream of cold water. Centrifuge and filter the supernatant liquid.

Reference solution. A 0.025 per cent w/v solution of *acamprosate impurity A IPRS* (*homotaurine IPRS*) in *water*. Dilute 0.4 ml of the solution to 100.0 ml with *borate buffer solution pH 10.4*. Treat 3.0 ml of the solution in the same way as the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with spherical octadecylsilane bonded to porous silica (5 µm) with a specific surface area of 170 m^2/g, and a pore size of 12 nm,
- mobile phase: a mixture of 10 volumes of *acetonitrile*, 10 volumes of *methanol* and 80 volumes of *0.1 M phosphate buffer pH 6.5*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 261 nm,
- injection volume: 20 µl.

The retention time of fluorescamine is about 4 minutes; acamprosate impurity A is about 8 minutes. Acamprosate is not detected by this system.

Inject the reference solution and the test solution. Run the chromatogram 6 times the retention time of acamprosate impurity A. In the chromatogram obtained with the test solution, the area of any peak corresponding to acamprosate impurity A is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.05 per cent).

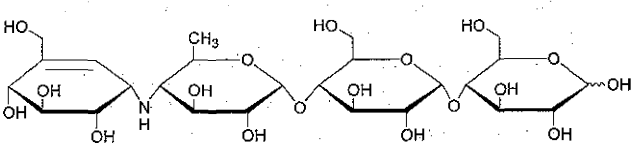
Heavy metals (2.3.13). Dissolve 2 g in *water* and dilute to 20 ml with *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D (10 ppm) using 10.0 ml of *lead standard solution (1 ppm)*.

Loss on drying (2.4.19). Not more than 0.4 per cent, determined on 1 g by drying in an oven at 105°.

Assay. To 4 g of *cation exchange resin* (75 to 150 µm) add 20 ml of *water* and stir magnetically for 10 minutes. Introduce this suspension into a glass column 45 cm x 2.2 cm, equipped with a polytetrafluoroethylene flow cap covered by a glass-wool plug. Allow a few ml of the solution to flow, then place a plug of glass wool over the resin. Pass 50 ml of *1 M hydrochloric acid* through the column. The eluate reaches to pH 1. Wash with 3 quantities, each of 200 ml, of *water* to obtain an eluate at pH 6. Dissolve 0.1 g of the substance under examination in 15 ml of *water*. Slowly pass through the column and wash with 3 quantities, each of 25 ml, of *water*, collecting the eluate. Allow to elute until an eluate at pH 6 is obtained. Titrate the solution obtained with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02002 g of $C_{10}H_{20}CaN_2O_8S_2$.

Acarbose



$C_{25}H_{43}NO_{18}$ Mol. Wt. 646.0

Acarbose is *O*-4,6-dideoxy-4-[[(1*S*,4*R*, 5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino-α-D-glucopyranosyl-(1→4)-*O*-α-D- glucopyranosyl-(1→4)-D-glucopyranose, which is produced by certain strains of *Actinoplanes utahensis*.

Acarbose contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{25}H_{43}NO_{18}$, calculated on the anhydrous basis.

Category. Antidiabetic.

Description. A white or yellowish, amorphous powder, hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acarbose IPRS* or with the reference spectrum of acarbose.

B. In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.5 to 7.5, determined in 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A).

Specific optical rotation (2.4.22). + 168° to + 183°, dilute 2 ml of solution A to 10 ml with *water*.

Light absorption (2.4.7). Absorbance of solution A at 425 nm, not more than 0.15.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution (a). A 0.6 per cent w/v solution of *acarbose impurity A IPRS (O-4,6–dideoxy-4-[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl) cyclohex-2-enyl] amino]-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-arabino-hex-2-ulopyranose IPRS)* in the test solution.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with aminopropylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 75 volumes of *acetonitrile*, 25 volumes of a solution containing 0.06 per cent w/v of *potassium dihydrogen orthophosphate* and 0.035 per cent w/v of *disodium hydrogen phosphate dihydrate*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the peak–to–valley ratio is not less than 1.2, where H_p is the height above the baseline of the peak due to acarbose impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to acarbose.

Inject reference solution (b) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to acarbose impurity A is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent), the area of the peak at relative retention time of about 0.5 is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the area of the peak at relative retention time of about 1.2 is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in *water* and dilute to 50.0 ml with *water*.

Reference solution. A 0.02 per cent w/v solution of *acarbose IPRS* in *water*.

Chromatographic system
- a stainless steel column 25 cm x 4 mm packed with aminopropylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of *acetonitrile* and 25 volumes of a solution containing 0.06 per cent w/v of *potassium dihydrogen phosphate* and 0.035 per cent w/v of *disodium hydrogen phosphate dihydrate*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{43}NO_{18}$.

Storage. Store protected from moisture.

Acarbose Tablets

Acarbose Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of acarbose, $C_{25}H_{43}NO_{18}$.

Usual strengths. 50 mg; 100 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 500 ml of a phosphate buffer prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* and 2 ml of *triethylamine* in 1000 ml of *water,* adjusted to pH 3.0 with *orthophosphoric acid,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 15 volumes of the phosphate buffer and 85 volumes of *acetonitrile.*

Test solution. Use the filtrate, dilute if necessary, with the solvent mixture to obtain a solution of 0.01 per cent w/v of Acarbose.

Reference solution. A 0.01 per cent w/v solution of *acarbose IPRS* in the solvent mixture.

Use the chromatographic system as described under Assay.

Calculate the content of $C_{25}H_{43}NO_{18}$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{25}H_{43}NO_{18}$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Acarbose in the mobile phase by shaking mechanically, dilute to 250.0 ml with the mobile phase and filter.

Reference solution. A 0.02 per cent w/v solution of *acarbose IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with amino groups bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 0.6 g of *potassium dihydrogen orthophosphate* and 0.35 g of *sodium dihydrogen phosphate* in 1000 ml of *water* and 60 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

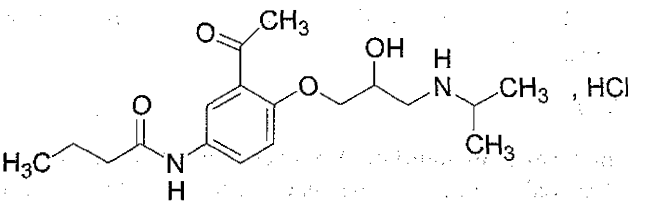
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{43}NO_{18}$ in the tablets.

Storage. Store protected from light and moisture.

Acebutolol Hydrochloride



$C_{18}H_{28}N_2O_4,HCl$ Mol. Wt. 372.9

Acebutolol Hydrochloride is (*RS*)-3'-acetyl-4'-(2-hydroxy-3-isopropylaminopropoxy)butyranilide hydrochloride.

Acebutolol Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{28}N_2O_4$, HCl, calculated on the dried basis.

Category. β_1-receptor antagonist; antihypertensive; antianginal; antiarrhythmic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acebutolol hydrochloride IPRS* or with the reference spectrum of acebutolol hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in 0.1 per cent v/v solution of *hydrochloric acid* shows absorption maxima at 233 nm 322 nm; absorbance at 233 nm, 0.55 to 0.61.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 60 volumes of *water*, 40 volumes of *methanol* and 0.5 volume of *perchloric acid.*

Test solution. Dissolve 0.1 g of the substance under examination in *methanol* and dilute to 100.0 ml with *methanol.*

Reference solution (a). A 0.1 per cent w/v solution of *acebutolol hydrochloride IPRS* in *methanol.*

Reference solution (b). A mixture of equal volumes of reference solution (a) and a 0.1 per cent w/v solution of *pindolol IPRS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

D. A 5 per cent w/v solution gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1) and not more intensely coloured than reference solution BYS5 (2.4.1).

pH (2.4.24). 5.0 to 7.0, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with mobile phase A.

Reference solution (c). Mix 2.0 ml of reference solution (b) and 1.0 ml of 0.2 per cent w/v solution of *acebutolol impurity A IPRS (N-[3-acetyl-4-[(2RS)-3-(ethylamino)-2-hydroxy-propoxy]phenyl] butanamide IPRS)* and dilute to 10.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. mix 2.0 ml of *orthophosphoric acid* and 3.0 ml of *triethylamine* and dilute to 1000 ml with *water,*

  B. equal volumes of *acetonitrile* and mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 25 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
2	98	2
30.5	10	90
41	10	90
42	98	2
50	98	2

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to acebutolol impurity A and acebutolol is not less than 7.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The sum of the areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.3 g in 50 ml of *ethanol (95 per cent)* and add 1 ml of *0.1 M hydrochloric acid.* Titrate with *0.1 M sodium hydroxide,* determining the end point potentiometrically (2.4.25). Read the volumes added between the two points of inflection.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03729 g of $C_{18}H_{28}N_2O_4,HCl$.

Storage. Store protected from light.

Acebutolol Tablets

Acebutolol Hydrochloride Tablets

Acebutolol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of acebutolol hydrochloride, $C_{18}H_{28}N_2O_4$, HCl.

Usual strengths. 200 mg; 400 mg.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the solution obtained in the Assay, shows an absorption maximum at 233 nm.

B. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *water*, 40 volumes of *methanol* and 0.5 volume of *perchloric acid*.

Test solution. Disperse a quantity of the powdered tablets containing 0.5 g of Acebutolol Hydrochloride with 30 ml of *methanol*, with the aid of ultrasound for 15 minutes and dilute to 50.0 ml with *methanol*, centrifuge and use the clear supernatant liquid.

Reference solution (a). A 0.1 per cent w/v solution of *acebutolol hydrochloride IPRS* in *methanol*.

Reference solution (b). A mixture of equal volumes of reference solution (a) and a 0.1 per cent w/v solution of *pindolol IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating two plates with *silica gel GF254*.

Mobile phase (a). The upper layer obtained by shaking together 50 volumes of *water*, 40 volumes of *1-butanol* and 10 volumes of *glacial acetic acid*.

Mobile phase (b). A mixture of 90 volumes of *2-propanol* and 10 volumes of *glacial acetic acid*.

Test solution. Disperse a quantity of the powdered tablets containing 0.5 g of Acebutolol Hydrochloride with 30 ml of *methanol*, with the aid of ultrasound for 15 minutes and dilute to 50.0 ml with *methanol*, centrifuge and use the clear supernatant liquid.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with *methanol*.

Reference solution (b). Dilute 3.0 ml of reference solution (a) to 100.0 ml with *methanol*.

Reference solution (c). Dilute 1.0 ml of the reference solution (a) to 100.0 ml with *methanol*.

Apply 20 µl of each solution on each plate. Develop two chromatograms using separately the two mobile phases. After development, dry the plates in a current of warm air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatograms obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than two such spots are more intense than the spot in the chromatograms obtained with reference solution (c). Ignore any spot at the point of application.

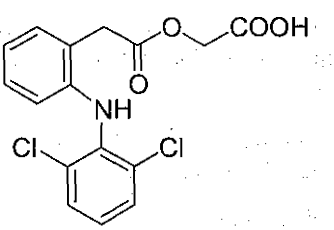
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of Acebutolol Hydrochloride with 40 ml of *0.1M hydrochloric acid* and add sufficient *water* to produce 100.0 ml, filter and dilute 10.0 ml of the filtrate to 100.0 ml with *water*. Dilute 10.0 ml of the solution to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 233 nm (2.4.7).

Calculate the content of $C_{18}H_{28}N_2O_4$,HCl taking 580 as the specific absorbance at 233 nm.

Storage. Store protected from light.

Aceclofenac



$C_{16}H_{13}Cl_2NO_4$ Mol. Wt. 354.2

Aceclofenac is 2-[(2,6-dichlorophenyl)amino] phenylacetoxyacetic acid.

Aceclofenac contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{13}Cl_2NO_4$, calculated on the dried basis.

Category. Nonsteroidal antiinflammatory.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aceclofenac IPRS* or with the reference spectrum of aceclofenac.

B. When examined in the range 220 nm to 370 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* shows an absorption maximum at 275 nm.

C. Dissolve 10 mg in 10 ml of *ethanol*. To 1 ml of the solution, add 0.2 ml of a mixture, prepared immediately before use, of equal volumes of a 0.6 per cent solution of *potassium ferricyanide* and a 0.9 per cent solution of *ferric chloride*. Allow to stand protected from light for 5 minutes. Add 3 ml of a 1 per cent solution of *hydrochloric acid*. Allow to stand protected from light for 15 minutes. A blue colour develops and a precipitate is formed.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE —Prepare the solutions immediately before use.

Solvent mixture. 30 volumes of mobile phase A and 70 volumes of mobile phase B.

Test solution. Dissolve 50 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution (a). A 0.043 per cent w/v solution of *aceclofenac impurity A IPRS (diclofenac sodium IPRS)* in the solvent mixture.

Reference solution (b). Mix 1.0 ml of reference solution (a) and 5.0 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Reference solution (c). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a 0.11 per cent v/v solution of *orthophosphoric acid*, adjusted to pH 7.0 with *sodium hydroxide solution*,
  B. a mixture of 10 volumes of *water* and 90 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
25	50	50
30	20	80
50	20	80
55	70	30

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to aceclofenac impurity A and aceclofenac is not less than 5.0.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to aceclofenac impurity A and any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.7 per cent). Ignore any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.02 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 45 volumes of *water* and 55 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *aceclofenac IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of buffer solution prepared by diluting 1 ml of *orthophosphoric acid* to 1000 ml with *water* and 55 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 20 µl.

The retention time of the principal peak is about 5.0 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{13}Cl_2NO_4$.

Storage. Store protected from light, at a temperature not exceeding 30°.

Aceclofenac Tablets

Aceclofenac Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aceclofenac, $C_{16}H_{13}Cl_2NO_4$.

Usual strength. 100 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium: 900 ml of *phosphate buffer pH 7.5*,
Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7). Calculate the content of aceclofenac, $C_{16}H_{13}Cl_2NO_4$ in the medium from the absorbance obtained from a solution of known concentration of *aceclofenac IPRS*.

Q. Not less than 70 per cent of the stated amount of $C_{16}H_{13}Cl_2NO_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and of *water*.

Test solution. Disperse a quantity of powdered tablets containing 100 mg of Aceclofenac with the solvent mixture anddilute to 100.0 ml with the solvent mixture, filter.

Reference solution (a). A 0.1 per cent w/v solution of *aceclofenac IPRS* in the solvent mixture.

Reference solution (b). Dissolve a quantity of *diclofenac sodium IPRS* containing 25 mg of diclofenac in the solvent mixture and dilute to 25.0 ml with the solvent mixture. Dilute 1.0 ml of the solution and 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with dimethyloctylsilane bonded to porous silica (5 µm) (Such as Hypersil MOS),
- mobile phase: a mixture of 55 volumes of buffer pH 3.5 prepared by adding 1.2 ml of *glacial acetic acid* in 1000 ml of *water*, adjusted to pH 3.5 with *dilute sodium hydroxide solution*, 22.5 volumes of *acetonitrile* and 22.5 volumes of *tetrahydrofuran*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to aceclofenac and diclofenac is not less than 5.0 and the column efficiency is not less than 2000 theoretical plates for peak due to aceclofenac.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of peak due to diclofenac is not more than 5 times the area of the peak due to diclofenac in the chromatogram obtained with reference solution (b) (5.0 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks other than diclofenac peak is not more than twice the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 55 volumes of *acetonitrile* and 45 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 100 mg of Aceclofenac with 60 ml of *acetonitrile*, with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with *acetonitrile*. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *aceclofenac IPRS* in *acetonitrile*. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

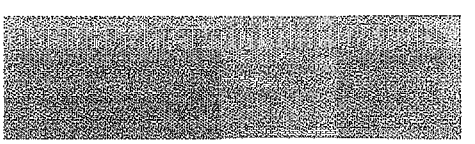
Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of 55 volumes of buffer solution prepared by adding 1.0 ml of *glacial acetic acid* in 1000 ml of *water* and 45 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

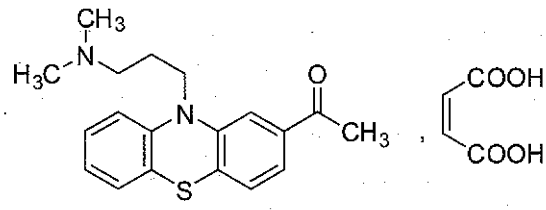
Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{13}Cl_2NO_4$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.



Acepromazine Maleate



$C_{19}H_{22}N_2OS,C_4H_4O_4$ Mol. Wt. 442.5

Acepromazine Maleate is 2-acetyl-10-(3-dimethylaminopropyl) phenothiazine hydrogen maleate.

Acepromazine Maleate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{19}H_{22}N_2OS,C_4H_4O_4$, calculated on the dried basis.

Category. Antipsychotic.

Description. A yellow coloured, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acepromazine maleate IPRS* or with the reference spectrum of acepromazine maleate.

B. Complies with the test for Identification of Phenothiazine (2.3.3).

C. Dissolve 0.2 g in a mixture of 3 ml of *water* and 2 ml of *5 M sodium hydroxide* and shake with 3 ml of *ether*. Add to the aqueous solution 2 ml of *bromine solution*, warm in a water-bath for 10 minutes, heat to boiling, cool and add 0.25 ml to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*; a bluish black colour develops on heating for 15 minutes in a water-bath.

Tests

pH (2.4.24). 4.0 to 5.5, determined in a 1.0 per cent w/v solution.

Related substances. Complies with the test for related substances in phenothiazines (2.3.5), but using a mixture of 75 volumes of *n-hexane*, 17 volumes of *butan-2-one* and 8 volumes of *diethylamine* as the mobile phase.

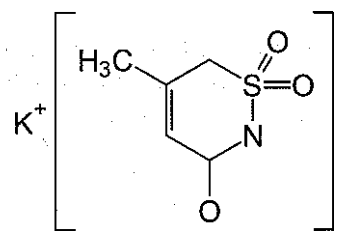
Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa for 16 hours.

Assay. Dissolve 0.4 g in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04425 g of $C_{19}H_{22}N_2OS,C_4H_4O_4$.

Acesulphame Potassium



$C_4H_4KNO_4S$ Mol. Wt. 201.2

Acesulphame Potassium is potassium 6-methyl-1,2,3-oxathiazin-4-olate 2,2-dioxide.

Acesulphame Potassium contains not less than 99.0 per cent and not more than 101.0 per cent of $C_4H_4KNO_4S$, calculated on the dried basis.

Category. Sweetening agent.

Description. A white or almost white, crystalline powder or colourless crystals.

Identification

Test B may be omitted if tests A and C are carried out and test A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *acesulphame potassium IPRS* or with the reference spectrum of acesulphame potassium.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F254*.

Mobile phase. A mixture of 10 volumes of *ammonia*, 60 volumes of *acetone* and 60 volumes of *ethyl acetate*.

Test solution. Dissolve 5 mg of the substance under examination in *water* and dilute to 5.0 ml with *water*.

Reference solution (a). A 0.1 per cent w/v solution of *acesulphame potassium IPRS* in *water*.

Reference solution (b). A solution containing 0.1 per cent w/v each of *acesulphame potassium IPRS* and *saccharin sodium* in *water*.

Apply to the plate 5 µl of each solution. Run the plate twice over a path of 15 cm. Dry the plate in warm air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solution

(a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. 0.5 ml of solution A gives reaction (A) of potassium salts (2.3.1).

Tests

Solution A. A 20 per cent w/v solution in *carbon dioxide-free water*.

Appearance of solution. Solution A is clear and colourless (2.4.1).

Acidity or alkalinity. To 20 ml of solution A, add 0.1 ml of *bromothymol blue solution*. Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Impurity A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 2 volumes of *water*, 15 volumes of *ethanol (95 per cent)* and 74 volumes of *ethyl acetate*.

Test solution. Dissolve 0.8 g of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution (a). A 0.2 per cent w/v solution of *acetylacetamide* (acesulphame impurity A) in *water*. To 5.0 ml of the solution, add 45 ml of *water* and dilute to 100.0 ml with *methanol*.

Reference solution (b). To 10.0 ml of reference solution (a), add 1.0 ml of the test solution and dilute to 20.0 ml with *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with *phosphoric vanillin solution* and heat at 120° for about 10 minutes. Any spot due to acesulphame impurity A is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.125 per cent). The chromatogram obtained with reference solution (a) shows a clearly visible spot and the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution (a). A 0.004 per cent w/v solution of *acesulphame potassium impurity B IPRS (5-chloro-6-methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide IPRS)* in *water*. Dilute 1.0 ml of the solution to 200.0 ml with *water*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *water*. Further dilute 1.0 ml of the solution to 10.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile*, 60 volumes of 0.33 per cent w/v solution of *tetrabutylammonium hydrogen sulphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 234 nm,
- injection volume: 20 µl.

The relative retention time with reference to acesulphame for acesulphame impurity B is about 1.6.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0 for the principal peak.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. The area of any peak corresponding to acesulphame impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (20 ppm), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) except for the peak due to acesulphame impurity B (0.05 per cent).

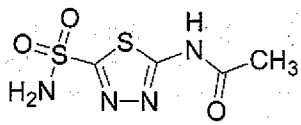
Heavy metals (2.3.13). 12 ml of solution A complies with the limit test for heavy metals, Method D (5 ppm), using 10.0 ml of *lead standard solution (1 ppm)*.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.15 g in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02012 g of $C_4H_4KNO_4S$.

Acetazolamide



$C_4H_6N_4O_3S_2$ Mol. Wt. 222.2

Acetazolamide is *N*-(5-sulphamoyl-1,3,4-thiadiazol-2-yl) acetamide.

Acetazolamide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_4H_6N_4O_3S_2$, calculated on the dried basis.

Category. Carbonic anhydrase inhibitor; used in the treatment of glaucoma.

Description. A white to faintly yellowish-white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acetazolamide IPRS* or with the reference spectrum of acetazolamide.

B. When examined in the range 230 nm to 260 nm (2.4.7), a 0.003 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum at 240 nm; absorbance at 240 nm, 0.49 to 0.53. When examined in the range 260 nm to 360 nm (2.4.7), a 0.00075 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum at 292 nm; absorbance at 292 nm, 0.43 to 0.47.

C. To about 20 mg in a test-tube add 4 ml of *2 M hydrochloric acid* and 0.2 g of *zinc powder* and immediately place a piece of *lead acetate paper* over the mouth of the tube; the paper exhibits a brownish-black colour.

D. To 25 mg, add 5 ml of *water*, 4 drops of *1 M sodium hydroxide* and 2 drops of *cupric sulphate solution*; a bluish-green colour or precipitate is produced.

Tests

Silver-reducing substances. Mix 5 g with 25 ml of *ethanol (95 per cent)*, add 125 ml of *water*, 10 ml of *nitric acid* and 5 ml of *0.1 M silver nitrate*, stir for 30 minutes and filter. Wash the residue with *water*, mix the filtrate and washings and titrate the excess of silver nitrate in the mixture with *0.05 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator; not less than 9.5 ml of *0.05 M ammonium thiocyanate* is required.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped propoxybenzene bonded to porous silica (4 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of 0.68 per cent w/v solution of *potassium dihydrogen phosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 25 µl.

Name	Relative retention time	Correction factor
Acetazolamide impurity E[1]	0.3	—
Acetazolamide impurity D[2]	0.4	1.6
Acetazolamide impurity B[3]	0.6	2.3
Acetazolamide (Retention time: about 8 minutes)	1.0	—
Acetazolamide impurity C[4]	1.4	2.6
Acetazolamide impurity A[5]	2.1	—
Acetazolamide impurity F[6]	2.6	—

[1]5-acetamido-1,3,4-thiadiazole-2-sulphonic acid,

[2]5-amino-1,3,4-thiadiazole-2-sulphonamide,

[3]*N*-(1,3,4-thiadiazol-2-yl)acetamide,

[4]*N*-(5-sulphanyl-1,3,4-thiadiazol-2-yl)acetamide,

[5]*N*-(5-chloro-1,3,4-thiadiazol-2-yl)acetamide,

[6]*N*-[5-[(5-acetamido-1,3,4-thiadiazol-2-yl)sulfonyl]sulphamoyl-1,3,4-thiadiazol-2- yl]acetamide.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 3.5 times the retention time of the principal peak. The area of any secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.15 per cent) and the sum of the areas of all the secondary peaks is not more than 6 times the area of the principal peak in the chromatogram obtained with the reference solution (0.6 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g dissolved in a mixture of 10 ml of *1 M sodium hydroxide* and 15 ml of *water* complies with the limit test for heavy metals, Method C (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the

mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.004 per cent w/v solution of *acetazolamide IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* in 1000 ml of *water* and 10 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

The retention time of the principal peak is about 5.5 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_4H_6N_4O_3S_2$.

Storage. Store protected from light.

Acetazolamide Tablets

Acetazolamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of acetazolamide, $C_4H_6N_4O_3S_2$.

Usual strength. 250 mg.

Identification

A. To a quantity of the powdered tablets containing 0.5 g of Acetazolamide, add 10 ml of *1 M sodium hydroxide*, shake thoroughly and filter. Neutralise the filtrate with *glacial acetic acid*, filter and dry the resulting precipitate at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acetazolamide IPRS* or with the reference spectrum of acetazolamide.

B. Triturate a quantity of the powdered tablets containing 0.5 g of Acetazolamide with a mixture of 5 ml of *water* and 1 ml of *1 M sodium hydroxide*, transfer to a test tube, add 0.2 g of *zinc powder*, add 0.5 ml of *hydrochloric acid* and immediately place a piece of *lead acetate paper* over the mouth of the tube; the paper exhibits a brownish-black colour.

C. To a quantity of the powdered tablets containing 25 mg of Acetazolamide add 5 ml of *water*, 3 drops of *1 M sodium hydroxide* and 2 drops of *cupric sulphate solution*; a bluish-green colour or precipitate is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium: 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 100 rpm for 60 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium, if necessary. Measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7). Calculate the content of acetazolamide, $C_4H_6N_4O_3S_2$ in the medium from the absorbance obtained from a solution of known concentration of *acetazolamide IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_4H_6N_4O_3S_2$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A freshly prepared mixture of 50 volumes of *2-propanol*, 30 volumes of *ethyl acetate* and 20 volumes of *strong ammonia solution*.

Solvent mixture. Equal volumes of *ethanol (95 per cent)* and *ethyl acetate*.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Acetazolamide in the solvent mixture and dilute to 10.0 ml with the solvent mixture, filter.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

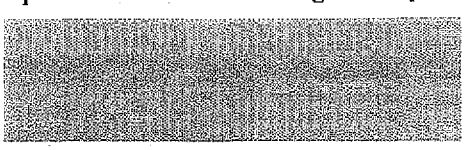
Apply to the plate 20 µl of each solution. Do not line the walls of the tank. Allow to saturate for 1 hour before development. After development, dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

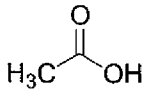
Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.4 g of Acetazolamide and add 90 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02222 g of $C_4H_6N_4O_3S_2$.

Storage. Store protected from light.



Glacial Acetic Acid



$C_2H_4O_2$ Mol. Wt. 60.1

Glacial Acetic acid contains not less than 99.0 per cent w/w and not more than 100.5 per cent w/w of $C_2H_4O_2$.

Category. Acidifying agent; buffering agent; pharmaceutical aid (analytical reagent).

Description. A crystalline mass or clear, colourless, volatile liquid.

Identification

A. A 10 per cent w/v solution is strongly acidic (2.4.46).

B. To 0.03 ml add 3 ml of *water* and neutralize with *2 M sodium hydroxide*; the solution gives reaction (C) of acetates (2.3.1).

Tests

Freezing point (2.4.11). Not less than 14.8°.

Residue on evaporation. Not more than 0.01 per cent, determined on 20.0 g by evaporating to dryness on a water-bath and drying at 105°.

Reducing substances. To 5 ml add 10 ml of *water* and mix. To 5 ml of the resulting solution add 6 ml of *sulphuric acid* and cool. Add 2 ml of *0.0167 M potassium dichromate*, allow to stand for 1 minute and add 25 ml of *water* and 1 ml of freshly prepared *dilute potassium iodide solution*. Titrate with *0.1 M sodium thiosulphate* using 1 ml of *starch solution* as indicator. Not less than 1.0 ml of *0.1 M sodium thiosulphate* is required.

Heavy metals (2.3.13). Dissolve the residue obtained in the test for Residue on evaporation by heating with two quantities, each of 15 ml, of *water* and add sufficient *water* to produce 50 ml (solution A). The solution complies with the limit test for heavy metals, Method D (5 ppm), using 10 ml of *lead standard solution (2 ppm Pb)*.

Iron (2.3.14). 5 ml of solution A diluted to 10 ml with *water* complies with the limit test for iron (5 ppm) Use 1.0 ml of *iron standard solution (10 ppm Fe)* to prepare the standard.

Chlorides (2.3.12). To 20 ml add sufficient *water* to produce 100 ml (solution B). 10 ml of solution B diluted to 15 ml with *water* complies with the limit test for chlorides (25 ppm). Use 10 ml of *chloride standard solution (5 ppm Cl)* to prepare the standard.

Sulphates (2.3.17). 15 ml of solution B complies with the limit test for sulphates (50 ppm).

Assay. Weigh a conical flask with a ground-glass stopper containing 25 ml of *water*, add 1 ml of the substance under examination and reweigh. Titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.06005 g of $C_2H_4O_2$.

Storage. Store protected from light and moisture.

Acetic Acid Ear Drops

Acetic Acid Otic Solution

Acetic Acid Ear Drops is a solution of Glacial Acetic Acid in a suitable non-aqueous solvent.

Acetic Acid Ear Drops contain not less than 85.0 per cent and not more than 130.0 per cent of the stated amount of acetic acid, $C_2H_4O_2$.

Usual strength. 2 per cent w/v.

Identification

A. Dilute 5 ml with 10 ml of *water* and adjusted to pH 7 with *1 M sodium hydroxide*. Add *ferric chloride test solution*, a deep red colour is produced, which is decolorized on the addition of *hydrochloric acid*.

B. Warm the solution with *sulphuric acid* and *ethanol (95 per cent)*; a characteristic odour of *ethyl acetate* is evolved.

Tests

pH (2.4.24). 2.0 to 4.0, determined in a 50.0 per cent v/v solution.

Other tests. Comply with the tests stated under Ear Drops.

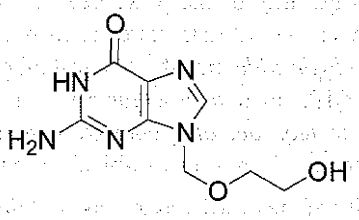
Assay. Transfer a volume containing 0.1 g of Glacial Acetic Acid to a conical flask, add 5 ml of *sodium chloride solution* and about 40 ml of *water*. Titrate with *0.1 M sodium hydroxide*, using 0.15 ml of *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.006005 g of $C_2H_4O_2$.

Storage. Store protected from light and moisture.

Aciclovir

Acyclovir



$C_8H_{11}N_5O_3$ Mol. Wt. 225.2

Aciclovir is 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one.

Aciclovir contains not less than 98.5 per cent and not more than 101.0 per cent of $C_8H_{11}N_5O_3$, calculated on the anhydrous basis.

Category. Antiviral.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aciclovir IPRS* or with the reference spectrum of aciclovir.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *0.1 M sodium hydroxide* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *dimethyl sulphoxide* and 80 volumes of *water*.

Test solution. Dissolve 25 mg of the substance under examination in 5 ml of *dimethyl sulphoxide* and dilute to 25.0 ml with *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b). A 0.01 per cent w/v solution, each of, *aciclovir IPRS* and *aciclovir impurity B IPRS* in *0.1 M sodium hydroxide*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 1 volume of *acetonitrile* and 99 volumes of a buffer solution prepared by dissolving 3.48 g of *dipotassium hydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.1 with *orthophosphoric acid*,

  B. a mixture of 50 volumes of *acetonitrile* and 50 volumes of a buffer solution prepared by dissolving 3.48 g of *dipotassium hydrogen phosphate* in 1000 ml of *water* and adjusted to pH 2.5 with *orthophosphoric acid*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
27	80	20
40	80	20
42	100	0
45	100	0

Name	Relative retention time	Correction factor
Aciclovir impurity B[1]	0.4	—
Aciclovir impurity P[2]	0.7	—
Aciclovir impurity C[3]	0.9	—
Aciclovir (Retention time: about 13 minutes)	1.0	—
Aciclovir impurity N[4]	1.37	—
Aciclovir impurity O[5]	1.42	—
Aciclovir impurity I[6]	1.57	1.5
Aciclovir impurity J[7]	1.62	—
Aciclovir impurity F[8]	1.7	—
Aciclovir impurity A[9]	1.8	—
Aciclovir impurity K[10]	2.5	—
Aciclovir impurity G[11]	2.6	—

[1]2-amino-1,7-dihydro-6*H*-purin-6-one (guanine),

[2]2-amino-9-(2-hydroxyethyl)1,9-dihydro-6*H*-purin-6-one

[3]2-amino-7-[(2-hydroxyethoxy)methyl]-1,7-dihydro-6*H*-purin-6-one,

[4]unknown structure,

[5]unknown structure,

[6]2-amino-7-[[2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl) methoxy]ethoxy]methyl]-1,7-dihydro-6*H*-purin-6-one,

[7]9,9'-[ethylenebis(oxymethylene)]bis(2-amino-1,9-dihydro-6*H*-purin-6-one),

[8]*N*-[9-[(2-hydroxyethoxy)methyl]-6-oxo-6, 9-dihydro-1*H*-purin-2-yl] acetamide,

[9]2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate,

[10]2,2'-[methylenediimino]bis[9-[(2-hydroxyethoxy)methyl]1,9-dihydro-6*H*-purin- 6-one],

[11]2-[[2-(acetylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy] ethyl acetate.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to aciclovir impurity B and aciclovir is not less than 20.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any peak due to aciclovir impurity B is not more than 7 times the area of the principal peak in the chromatogram obtained with

reference solution (0.7 per cent), the area of any other secondary peak is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.3 per cent) and the sum of the areas of all the secondary peaks is not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.5 g.

Assay. Dissolve 0.15 g, in 60 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02252 g of $C_8H_{11}N_5O_3$.

Aciclovir intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per mg of aciclovir.

Storage. Store protected from light and moisture.

Aciclovir Cream

Acyclovir Cream

Aciclovir Cream contains Aciclovir in a suitable cream base.

Aciclovir Cream contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, $C_8H_{11}N_5O_3$.

Usual strength. 5 per cent w/w.

Identification

A. When examined in the range 230 nm to 350 nm (2.4.7), the solution prepared in the Assay shows absorption maximum at 255 nm and shoulder at 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to the principal spot in the chromatogram obtained with reference solution (a).

Tests

Guanine. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F254* (Such as Merck cellulose F plates).

Mobile phase. Ethyl acetate.

Test solution (a). Disperse a quantity of cream containing 30 mg of Aciclovir with 3 ml of *0.1 M sodium hydroxide* into a 10-ml graduated stoppered centrifuge tube. Add 5 ml of a mixture of 1 volume of *chloroform* and 2 volumes of *propan-1-ol*, shake, centrifuge and dilute the upper aqueous layer to 5 ml with *0.1 M sodium hydroxide*. Mix, centrifuge and use the upper aqueous layer.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *0.1 M sodium hydroxide*.

Reference solution (a). A 0.06 per cent w/v solution of *aciclovir IPRS* in *0.1 M sodium hydroxide*.

Reference solution (b). A 0.006 per cent w/v solution of *guanine* in *0.1 M sodium hydroxide*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise to the top of the plate. Dry the plate in air and repeat the development in the same direction using a mixture of 10 volumes of *propan-1-ol*, 30 volumes of *13.5 M ammonia* and 60 volumes of a 5 per cent w/v solution of *ammonium sulphate* as the mobile phase. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any spot that appears just below the solvent front.

Other tests. Comply with the tests stated under Creams.

Assay. Disperse a quantity of cream containing 7.5 mg of Aciclovir with 50 ml of *0.5 M sulphuric acid*. Shake with 50 ml of *ethyl acetate*, allow to separate and collect the clear lower aqueous layer. Wash the organic layer with 20 ml of *0.5 M sulphuric acid* and dilute the combined washings and the aqueous layer to 100.0 ml with *0.5 M sulphuric acid*, mix and filter. Discard the first few ml of filtrate and to 10.0 ml of the filtrate add *water* to produce 50.0 ml. Measure the absorbance of the resulting solution at 255 nm (2.4.7). Calculate the content of $C_8H_{11}N_5O_3$, taking 562 as specific absorbance at the maximum at 255 nm.

Aciclovir Dispersible Tablets

Acyclovir Dispersible Tablets

Aciclovir Dispersible Tablets contain Aciclovir in a suitable dispersible base.

Aciclovir Dispersible Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, $C_8H_{11}N_5O_3$.

Usual strengths. 200 mg; 400 mg; 800 mg.

Identification

A. When examined in the range 230 nm to 350 nm (2.4.7), the solution prepared in the Assay shows an absorption maximum at 255 nm and a broad shoulder at 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Guanine. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F254*.

Mobile phase. A mixture of 10 volumes of *propan-1-ol*, 30 volumes of *13.5 M ammonia* and 60 volumes of a 5.0 per cent w/v solution of *ammonium sulphate*.

Test solution (a). Disperse a quantity of the powdered tablets containing 0.25 g of Aciclovir with 25 ml of *0.1 M sodium hydroxide* for 10 minutes and dilute to 50.0 ml with *0.1 M sodium hydroxide*, filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *0.1 M sodium hydroxide*.

Reference solution (a). A 0.05 per cent w/v solution of *aciclovir IPRS* in *0.1 M sodium hydroxide*.

Reference solution (b). A 0.005 per cent w/v solution of *guanine* in *0.1 M sodium hydroxide*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2 volumes of *13.5 M ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

Test solution. Disperse a quantity of the powdered tablets containing 0.25 g of Aciclovir with 10.0 ml of *dimethyl sulphoxide* for 15 minutes and filter.

Reference solution. Dilute 0.7 ml of the test solution to 100.0 ml with *dimethyl sulphoxide*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 10 cm, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.7 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.1 g of Aciclovir in 60 ml of *0.1 M sodium hydroxide* with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with *0.1 M sodium hydroxide* and filter. To 15 ml of the filtrate, add 50 ml of *water*, 5.8 ml of *2 M hydrochloric acid* and dilute to 100.0 ml with *water*. Dilute 5.0 ml of the solution to 50.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance at the maximum at 255 nm (2.4.7). Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the specific absorbance at the maximum at 255 nm.

Labelling. The label states that the tablets should be dispersed in water immediately before use.

Aciclovir Eye Ointment

Acyclovir Eye Ointment

Aciclovir Eye Ointment is a sterile preparation containing Aciclovir in a suitable base.

Aciclovir Eye Ointment contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, $C_8H_{11}N_5O_3$.

Usual strengths. 3 per cent w/w; 5 per cent w/w.

Identification

A. When examined in the range 230 nm to 350 nm (2.4.7), the solution prepared in the Assay shows an absorption maximum at 255 nm and a broad shoulder at 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Guanine. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F254*.

Mobile phase. A mixture of 10 volumes of *propan-1-ol*, 30 volumes of *13.5 M ammonia* and 60 volumes of a 5.0 per cent w/v solution of *ammonium sulphate*.

Test solution (a). Disperse a quantity of the ointment containing 25 mg of Aciclovir in 100 ml of *hexane*, extract with 5 ml of *0.1 M sodium hydroxide*, allow to separate and retain the lower aqueous layer.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *0.1 M sodium hydroxide*.

Reference solution (a). A 0.05 per cent w/v solution of *aciclovir IPRS* in *0.1 M sodium hydroxide*.

Reference solution (b). A 0.005 per cent w/v solution of *guanine* in *0.1 M sodium hydroxide*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

Other tests. Comply with the tests stated under Eye Ointment.

Assay. Disperse a quantity of the eye ointment containing 10 mg of Aciclovir in 60 ml of *hexane*. Extract with three 30-ml quantities of *0.1 M sodium hydroxide*, add sufficient *0.1 M sodium hydroxide* to produce 100 ml and filter. To 15.0 ml of the solution, add 5 ml of *2 M hydrochloric acid* and dilute to 100.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at 255 nm (2.4.7). Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the specific absorbance at 255 nm.

Aciclovir Intravenous Infusion

Acyclovir Intravenous Infusion; Acyclovir Sodium Intravenous Infusion

Aciclovir Intravenous Infusion is a sterile material consisting of aciclovir sodium, prepared from Aciclovir with the aid of a suitable alkali, with or without auxiliary substances. It is filled in a sealed container.

The infusion is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Aciclovir Intravenous Infusion contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, $C_8H_{11}N_5O_3$.

Usual strength. 500 mg per vial.

Description. A white or almost white, crystalline powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injections) and with the following requirements.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution prepared in the Assay shows an absorption maximum at 255 nm and a broad shoulder at 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. It gives reaction (A) of sodium salts (2.3.1).

Tests

Appearance of solution. Dissolve the contents of a sealed container in sufficient *Water for Injections* to produce a solution containing 2.5 per cent w/v solution of Aciclovir (solution A). The solution is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

pH (2.4.24). 10.7 to 11.7, determined in solution A.

Guanine. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F254* (Merck celllulose F plates are suitable).

Mobile phase. A mixture of 10 volumes of *1-propanol*, 30 volumes of *strong ammonia solution* and 60 volumes of a 5 per cent w/v solution of *ammonium sulphate*.

Test solution (a). Dissolve a suitable quantity of the substance under examination in sufficient *0.1 M sodium hydroxide* to produce a solution containing 0.5 per cent of Aciclovir.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *0.1 M sodium hydroxide*.

Reference solution (a). A 0.05 per cent w/v solution of *aciclovir IPRS* in *0.1 M sodium hydroxide*.

Reference solution (b). A 0.005 per cent w/v solution of *guanine* in *0.1 M sodium hydroxide*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the pate in a current of warm air and examine under ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 80 volumes of *dichloromethane*, 20 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

NOTE— Prepare the following solutions immediately before use.

Test solution. Dissolve a quantity of the substance under examination in *dimethyl sulphoxide* to produce a solution containing 2.5 per cent w/v of aciclovir.

Reference solution. Dilute 1 volume of the test solution to 200 volumes with *dimethyl sulphoxide*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. Any secondary spot with an R_f value greater than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (0.5 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.174 Endotoxin Units per mg of aciclovir.

Assay. Dissolve a quantity of the mixed contents of 10 containers containing 0.10 g of Aciclovir in sufficient *0.1 M hydrochloric acid* to produce 500.0 ml. Dilute 5.0 ml of the resulting solution to 100.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 255 nm (2.4.7). Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the specific absorbance at 255 nm.

Storage. Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°.

Labelling. The label states (1) the quantity of aciclovir sodium in the sealed container in terms of the equivalent amount of Aciclovir; (2) the strength of the constituted solution in terms of the equivalent amount of Aciclovir in a suitable dose-volume.

Aciclovir Oral Suspension

Acyclovir Oral Suspension

Aciclovir Oral Suspension is a suspension of Aciclovir in a suitable flavoured vehicle.

Aciclovir Oral Suspension contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, $C_8H_{11}N_5O_3$.

Usual strengths. 200 mg per 5 ml; 400 mg per 5 ml.

Identification

A. When examined in the range 230 nm to 250 nm (2.4.7), the solution prepared in the Assay before the final dilution shows maximum absorption at 255 nm and shoulder at 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to the principal spot in the chromatogram obtained with reference solution (a). If the R_f values of the principal spot in the chromatogram obtained with test solution (b) and reference solution (a) are different, the oral suspension complies if the chromatogram obtained with reference solution (c) shows a single, compact spot.

Tests

pH (2.4.24). 4.0 to 7.0.

Guanine. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F254* (Such as Merck cellulose F plates).

Mobile phase. A mixture of 10 volumes of *propan-1-ol*, 30 volumes of *13.5 M ammonia* and 60 volumes of a 5 per cent w/v solution of *ammonium sulphate*.

Solvent mixture. 35 volumes of *0.1 M sodium hydroxide* and 65 volumes of *ethanol*.

Test solution (a). Disperse a quantity of oral suspension containing 0.6 g of Aciclovir in 20 ml of *0.1 M sodium hydroxide* and dilute to 100.0 ml with *ethanol*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture.

Reference solution (a). A 0.06 per cent w/v solution of *aciclovir IPRS* in the solvent mixture.

Reference solution (b). A 0.006 per cent w/v solution of *guanine* in the solvent mixture.

Reference solution (c). A mixture of equal volumes of test solution (b) and reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any spot that appears just below the solvent peak.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Disperse a quantity of oral suspension containing 0.4 g of Aciclovir in 400 ml of *water* and 25 ml of *1 M sulphuric acid* with the aid of ultrasound for 10 minutes and dilute to 500 ml with *water*. Filter the solution, discard the first few ml of filtrate and dilute 5.0 ml of the filtrate to 200.0 ml with *0.05 M sulphuric acid*. Add 10 ml of the solution to 5 ml of a 0.01 per cent w/v solution of *cetrimide* in *0.05 M sulphuric acid*, add sufficient *0.05 M sulphuric acid* to produce 100.0 ml and measure the fluorescence (2.4.5), using an excitation wavelength of 308 nm and an emission wavelength of 415 nm. Set the instrument to zero using a 0.0005 per cent w/v solution of *cetrimide* in *0.05 M sulphuric acid*. Calculate the content of $C_8H_{11}N_5O_3$ in the oral suspension from the fluorescence obtained by carrying out the operation at the same time using a mixture prepared by adding 10.0 ml of a 0.002 per cent w/v solution of *aciclovir IPRS* in *0.05 M sulphuric acid* and beginning at the words "to 5 ml of a 0.01 per cent w/v solution of *cetrimide* ...". Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_8H_{11}N_5O_3$, weight in volume, using the declared content of $C_8H_{11}N_5O_3$ in *aciclovir IPRS*.

Aciclovir Tablets

Acyclovir Tablets

Aciclovir Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aciclovir, $C_8H_{11}N_5O_3$.

Usual strengths. *200 mg; 400 mg; 800 mg.*

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution prepared in the Assay shows an absorption maximum at 255 nm and a broad shoulder at 274 nm.

B. In the test for Guanine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium: 900 ml of *0. 1 M hydrochloric acid*,
Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium, if necessary. Measure the absorbance of the resulting solution at the maximum at about 255 nm (2.4.7). Calculate the content of aciclovir, $C_8H_{11}N_5O_3$ in the medium from the absorbance obtained from a solution of known concentration of *aciclovir IPRS* in the dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_8H_{11}N_5O_3$.

Guanine. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F254* (Such as Merck celllulose F plates).

Mobile phase. A mixture of 10 volumes of *1- propanol*, 30 volumes of *strong ammonia solution* and 60 volumes of a 5 per cent w/v solution of *ammonium sulphate*.

Test solution (a). Disperse a quantity of the powdered tablets containing 0.25 g of Aciclovir with 25 ml of *0.1 M sodium hydroxide* with the aid of ultrasound for 10 minutes and dilute to 50.0 ml with *0.1 M sodium hydroxide*. Allow to stand and allow any undissolved material to settle before application to the plate.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *0.1 M sodium hydroxide*.

Reference solution (a). A 0.05 per cent w/v solution of *aciclovir IPRS* in *0.1 M sodium hydroxide*.

Reference solution (b). A 0.005 per cent w/v solution of *guanine* in *0.1 M sodium hydroxide*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. Any secondary spot corresponding to guanine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 80 volumes of *dichloromethane*, 20 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

NOTE— Prepare the solutions immediately before use.

Test solution. Disperse a quantity of the powdered tablets containing 0.25 g of Aciclovir in *dimethyl sulphoxide* and dilute to 10.0 ml with *dimethyl sulphoxide*, with the aid of ultrasound for 15 minutes and filter.

Reference solution. Dilute 0.7 ml of the test solution to 100.0 ml with *dimethyl sulphoxide*.

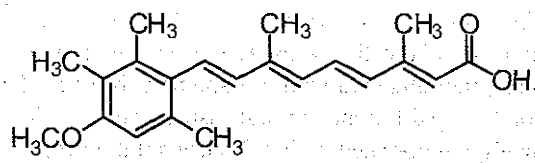
Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. Any secondary spot with an R_f value more than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.7 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.1 g of Aciclovir in 60 ml of *0.1 M sodium hydroxide* with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with *0.1 M sodium hydroxide*, filter. To 15.0 ml of the filtrate add 50 ml of *water*, 5.8 ml of *2 M hydrochloric acid* and sufficient *water* to produce 100.0 ml. To 5.0 ml of the solution add sufficient *0.1 M hydrochloric acid* to produce 50.0 ml and mix well. Measure the absorbance of the solution at the maximum at about 255 nm (2.4.7), using *0.1 M hydrochloric* acid as the blank. Calculate the content of $C_8H_{11}N_5O_3$ taking 560 as the specific absorbance at 255 nm.

Storage. Store protected from light.

Acitretin



$C_{21}H_{26}O_3$ Mol. Wt. 326.4

Acitretin is (2*E*,4*E*,6*E*,8*E*)-9-(4-Methoxy-2,3, 6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid.

Acitretin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{26}O_3$, calculated on the dried basis.

Category. Antipsoriatic.

Description. A yellow or greenish yellow crystalline powder. It shows polymorphism (2.5.11).

Identification

Test A may be omitted if test B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acitretin IPRS* or with the reference spectrum of acitretin.

B. When examined in the range 300 nm to 400 nm (2.4.7), a 0.000375 per cent w/v solution in *tetrahydrofuran* shows absorption maxima at 358 nm and specific absorbance at the absorption maxima is 1350 to 1475.

C. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light, and prepare solutions immediately before use.

Test solution (a). Dissolve 25 mg of the substance under examination in 5 ml of *tetrahydrofuan* and dilute immediately to 100.0 ml with *ethanol.*

Test solution (b). Dilute 10.0 ml of test solution (a) to 25.0 ml with *ethanol.*

Reference solution (a). Dissolve 25 mg of *acitretin IPRS* in 5 ml of *tetrahydrofuran* and dilute immediately to 100.0 ml with *ethanol.* Dilute 10.0 ml of the solution to 25.0 ml with *ethanol.*

Reference solution (b). Dissolve 1 mg of *tretinoin IPRS* in *ethanol* and dilute to 20.0 ml with *ethanol.* Mix 5.0 ml of the solution with 2.5 ml of reference solution (a) and dilute to 100.0 ml with *ethanol.*

Reference solution (c). Dilute 2.5 ml of reference solution (a) to 50.0 ml with *ethanol.* Dilute 3.0 ml of the solution to 20.0 ml with *ethanol.*

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Lichrospher PAH, Merck),
- sample temperature: 4°,
- mobile phase: a 0.3 per cent v/v solution of *glacial acetic acid* in a mixture of 8 volumes of *water* and 92 volumes of *ethanol,*
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 360 nm,
- injection volume: 10 µl.

Name	Relative retention time (in minutes)
Acetretin impurity A[1]	0.78
Tretinoin	0.84
Acetretin	1.0
Acetretin impurity B[2]	1.65

[1](2*Z*,4*E*,6*E*,8*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8- tetraenoic acid,

[2]ethyl (all-*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-nona-2,4,6,8- tetraenoate.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to acitretin and tretinion is not less than 2.0.

Inject reference solution (b), (c) and test solution (a). Run the chromatogram 2.5 times the retention time of principal peak. In the chromatogram obtained with test solution (a), the area of any secondary peak due to acitretin impurities A and B is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent). The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c).

Palladium. Not more than 10 ppm. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 247.6 nm using air-acetylene flame and Palladium hollow-cathode lamp.

Test solution. Take 2.0 g into a quartz beaker and add 3 ml of *magnesium nitrate solution.* Heat in a muffle furnace at 350° to incinerate the content. Ignite at about 450° for 8 hours and then at 550 ± 50° for a further one hour. Dissolve the residue in a mixture of 0.75 ml of *hydrochloric acid* and 0.25 ml of *nitric acid,* warming gently. Cool, then transfer the solution into a volumetric flask containing *water* and dilute to 50.0 ml with *water.*

Reference solution. Dissolve 0.163 g of *heavy magnesium oxide* in a mixture of 0.5 ml of *nitric acid,* 1.5 ml of *hydrochloric acid* and 50 ml of *water,* add 2.0 ml of *palladium standard solution (20 ppm Pd)* and dilute to 100.0 ml with *water.*

Heavy metals (2.3.13). 1.0 g complies with the test for heavy metals, Method B (20 ppm).

Sulphated ash (2.4.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 100° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and test solution (b).

Calculate the content of $C_{21}H_{26}O_3$.

Storage. Store protected from light and moisture, at a temperature 2° to 8°. It is recommended that the contents of an opened container be used as soon as possible and any unused part be protected by an atmosphere of inert gas.

Acitretin Capsules

Acitretin Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of acitretin, $C_{21}H_{26}O_3$.

NOTE — Carry out the following tests avoiding exposure to actinic light and using freshly prepared solutions.

Usual strengths. 10 mg; 25 mg.

Identification

A. Dissolve a quantity of capsule contents containing 25 mg of Acitretin in *methanol* and dilute to 250.0 ml with *methanol*, filter. Dilute 1.0 ml of the filtrate to 20.0 ml with *methanol*. When examined in the range 230 nm to 500 nm (2.4.7), the solution shows an absorption maximum at 346 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of 3 per cent w/v solution of *sodium lauryl sulphate*, adjusted to pH 9.5 with *0.01M hydrochloric acid* or *0.01M sodium hydroxide*,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted to obtain 0.0005 per cent w/v of acitretin, at the maximum at about 348 nm (2.4.7). Calculate the content of $C_{21}H_{26}O_3$ in the medium from the absorbance obtained from a solution of known concentration of *acitretin IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{26}O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 10 volumes of *tetrahydrofuran* and 13 volumes of *methanol*.

Test solution. Disperse a quantity of the capsule contents containing 25 mg of Acitretin with 8 ml of *water* in a water-bath at 45° for 10 minutes. Mix with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the solvent mixture, filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Further dilute 4.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.00025 per cent w/v each of *tretinoin IPRS* and *acitretin IPRS* in the solvent mixture.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 4.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.5 volume of *glacial acetic acid*, 5 volumes of *ethanol*, 21 volumes of *water* and 74 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 365 nm,
- injection volume: 10 µl.

Inject reference solution (b) and (c). The test is not valid unless in the chromatogram obtained with reference solution (b), the resolution between the peaks due to acitretin and tretinoin is not less than 3.0 and in the chromatogram obtained with reference solution (c), the signal-to-noise ratio of the principal peak is not less than 10.

Inject reference solution (a), (c) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) except the area of one secondary peak is more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 10 volumes of *tetrahydrofuran* and 13 volumes of *methanol*.

Test solution. Disperse a quantity of the mixed contents of 20 capsules containing 25 mg of Acitretin with 8 ml of *water* in a water-bath at 45° for 10 minutes. Mix with the aid of

ultrasound for 15 minutes and dilute to 100.0 ml with the solvent mixture. Further dilute 5.0 ml of the solution to 25.0 ml with the solvent mixture and filter.

Reference solution (a). A 0.005 per cent w/v solution of *acitretin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.00025 per cent w/v, each of, *acitretin IPRS* and *tretinoin IPRS* in the solvent mixture.

Use chromatographic system as described under Related substances.

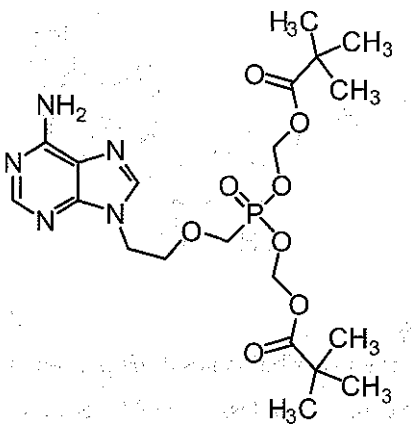
Inject reference solution (b).The test is not valid unless the resolution between the peaks due to tretinoin and acitretin is not less than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{21}H_{26}O_3$ in the capsules.

Storage. Store protected from light.

Adefovir Dipivoxil



$C_{20}H_{32}N_5O_8P$ Mol. Wt. 501.5

Adefovir Dipivoxil is 9-[2-bis[(pivaloyloxy)methoxy] phosphonyl]-methoxy]ethyl]adenine.

Adefovir Dipivoxil contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{32}N_5O_8P$, calculated on the dried basis.

Category. Antiretroviral.

Description. A white to off-white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with that of *adefovir dipivoxil IPRS* or with the reference spectrum of adefovir dipivoxil.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.001 per cent w/v solution of *adefovir dipivoxil IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with strong-cation exchange packing-sulphonated fluorocarbon polymer bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. *0.05 M sodium dihydrogen phosphate* in 0.1 per cent v/v *triethylamine*. To 1000 ml of the solution, add 1 g *hexane sulphonate*, adjusted to pH 3.3 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
12	80	20
47	40	60
57	40	60
65	80	20

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent). Ignore any peak with an area less than 0.01 times the area of the principal peak in the chromatogram obtained with the reference solution (0.01 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 60° for 3 hours.

Assay. Weigh 0.3 g, dissolve in 40 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.050147 g of $C_{20}H_{32}N_5O_8P$.

Adefovir Tablets

Adefovir Dipivoxil Tablets

Adefovir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of adefovir dipivoxil, $C_{20}H_{32}N_5O_8P$.

Usual strength. 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.03 per cent w/v solution of *adefovir dipivoxil IPRS* in the mobile phase. Further dilute 4.0 ml of the solution to 100.0 ml with the dissolution medium.

Use chromatographic system as described under Assay.

Calculate the content of $C_{20}H_{32}N_5O_8P$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{20}H_{32}N_5O_8P$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in 30 ml of the mobile phase, with the aid of ultrasound for 20 minutes and dilute to 50.0 ml with the mobile phase. Centrifuge and use the supernatant liquid.

Calculate the content of $C_{20}H_{32}N_5O_8P$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of Adefovir Dipivoxil in 15 ml of the mobile phase and dilute to 50.0 ml with the mobile phase and centrifuge. Dilute 5.0 ml of the supernatant liquid to 25.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *adefovir dipivoxil IPRS* in the mobile phase.

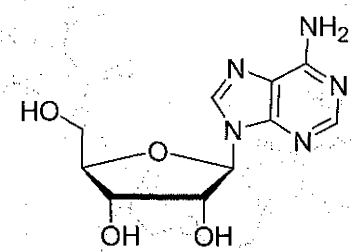
Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 8.7 g of *dipotassium hydrogen orthophosphate* and 0.15 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, 25 volumes of *acetonitrile* and 25 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{32}N_5O_8P$ in the tablets.

Adenosine



$C_{10}H_{13}N_5O_4$ Mol. Wt. 267.2

Adenosine is 9-β-D-ribofuranosyl-9*H*-purin-6-amine.

Adenosine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{13}N_5O_4$, calculated on the dried basis.

Category. Antiepileptic.

Description. A white or off white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *adenosine IPRS* or with the reference spectrum of adenosine.

Tests

Appearance of solution. A 5.0 per cent w/v solution in hot *water* (Solution A) is colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A, add 0.1 ml of *bromocresol purple solution* and 0.1 ml of *0.01 M hydrochloric*

acid. The solution is yellow. Add 0.4 ml of *0.01 M sodium hydroxide*. The solution is violet-blue.

Specific optical rotation (2.4.22). −49° to −45°, determined in a freshly prepared 2.5 per cent w/v solution in *1 M hydrochloric acid*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of *potassium hydrogen sulphate* and 3.4 g of *tetrabutylammonium hydrogen sulphate* in *water*, adjusted to pH 6.5 with 6.0 per cent w/v solution of *potassium hydroxide* and dilute to 1000 ml with the same solvent. Use freshly prepared solvent mixture.

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg each of adenosine impurities A and G in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 4.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *water* and 60 volumes of solvent mixture,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Adenosine impurity A[1]	0.3	0.6
Adenosine impurity G[2]	0.4	1.4
Adenosine	1.0	---

[1]adenine,

[2]inosine.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to adenosine impurities A and G is not less than 1.5.

Inject reference solution (a) and the test solution. Run the chromatogram 1.5 times the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to adenosine impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of any peak due to adenosine impurity G is not more than area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than the 5 times area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with a n area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (2.3.12). Dissolve 2.5 g in *water* and dilute to 25 ml with *water*. The solution complies with the limit test for chlorides (100 ppm).

Sulphates (2.3.17). 15 ml of solution A, complies with the limit for sulphates (200 ppm).

Ammonium (2.3.53). Not more than 10 ppm,using method B, determined on 0.5 g. Prepare the reference solution using 5 ml of *ammonium standard solution* (1 ppm NH_4).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g. by drying in an oven at 105°.

Assay. Weigh 0.2 g, dissolve in a mixture of 20 ml of *acetic anhydride* and 30 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02672 g of $C_{10}H_{13}N_5O_4$.

Adenosine Injection

Adenosine Injection is a sterile solution of Adenosine in Water for Injection. It may contain Sodium Chloride.

Adenosine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of adenosine, $C_{10}H_{13}N_5O_4$.

Usual strength. 3 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

Tests

pH (2.4.24). 4.5 to 7.5.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay.

Inject test solution (a). The area of any secondary peak is not more than 1.0 per cent and the sum of areas of all the secondary peak is not more than 1.5 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 11.62 Endotoxin Units per mg of adenosine *(for rapid intravenous injection)* and not more than 5.95 Endotoxin Units per mg of adenosine *(for continuous peripheral intravenous infusion)*.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Dilute a volume of injection containing 30 mg of Adenosine to 100.0 ml with *water*.

Test solution (b). Dilute 5.0 ml of test solution (a) to 50.0 ml with *water*.

Reference solution (a). A solution containing 0.003 per cent w/v each of *adenosine IPRS* and *inosine* in warm *water* (50° to 55°).

Reference solution (b). A 0.003 per cent w/v solution of *adenosine IPRS* in warm *water* (50° to 55°). If sodium chloride is present in the injection, add 0.01 ml of sodium chloride solution (0.9 in 100) per ml of the anticipated final volume of the solution before addition of the warm *water*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 2.0 g of *monobasic potassium phosphate* in 800 ml of *water*, add 5 ml of *1.0 M tetrabutylammonium dihydrogen phosphate solution*, dilute with *water* to 980 ml and mix. Add 20 ml of *acetonitrile*,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

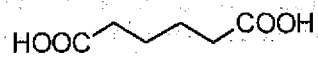
Inject reference solution (a) and (b). Run the chromatogram 2.5 times the retention time of the principal peak. The test is not valid unless in the chromatogram obtained with reference solution (a), the tailing factor for the principal peak is not more than 2.0 and the resolution between the peaks due to adenosine and inosine is not less than 6.0 and in the chromatogram obtained with reference solution (b), the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject reference solution (b) and test solution (b).

Calculate the content of $C_{10}H_{13}N_5O_4$ in the injection.

Storage. Store protected from moisture, in single dose containers, preferably of Type I glass.

Adipic Acid



$C_6H_{10}O_4$ Mol. Wt. 146.1

Adipic Acid is 1,6-Hexadioic acid.

Adipic Acid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_{10}O_4$, calculated on the dried basis.

Category. Pharmaceutical aid.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *adipic acid IPRS* or with the reference spectrum of adipic acid.

Tests

Solution A. Dissolve 5.0 g with heating in *water* and dilute to 50 ml with *water*. Allow to cool and to crystallise. Filter through a sintered-glass filter (G4). Wash the filter with *water*. Collect the filtrate and the washings until a volume of 50 ml is obtained.

Appearance of solution. A 5.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

Related substances. Determined by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve 20 mg of *glutaric acid* in 1.0 ml of the test solution and dilute to 10.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 3 volumes of *acetonitrile* and 97 volumes of a 2.5 per cent w/v solution of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 209 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peak corresponding to glutaric acid and adipic acid not less than 9.0.

Inject reference solution (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (2.3.12). Dilute 12.5 ml of solution A to 15 ml with *water* complies with the limit test for chlorides (200 ppm).

Nitrates. Not more than 30 ppm.

To 1 ml of solution A, add 2 ml of *13.5 M ammonia*, 0.5 ml of a 1 per cent w/v solution of *manganese sulphate*, 1 ml of a 1.0 per cent w/v solution of *sulphanilamide* and dilute to 20 ml with *water*. Add 0.1 g of *zinc powder* and cool in iced water for 30 minutes, shake from time to time. Filter and cool 10 ml of the filtrate in iced water. Add 2.5 ml of 25 per cent w/v solution of *hydrochloric acid* and 1 ml of a 1.0 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*. Allow to stand at room temperature. After 15 minutes, the mixture is not more intensely coloured than a standard prepared at the same time and in the same manner, using 1.5 ml of *nitrate standard solution* (2 ppm) instead of 1 ml of solution A. The test is not valid if a blank solution prepared at the same time and in the same manner, using 1 ml of *water* instead of 1 ml of solution A, is more intensely coloured than 0.0002 per cent w/v solution of *potassium permanganate*.

Sulphates (2.3.17). 3 ml of solution A complies with the limit test for sulphates (500 ppm).

Iron (2.3.14). 10 ml of solution A complies with the limit test for iron (10 ppm), using 1.0 ml of *iron standard solution (10 ppm)*.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

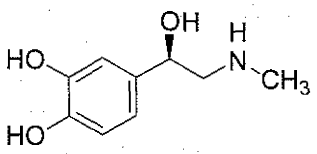
Loss on drying (2.4.19). Not more than 0.2 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 60 mg in 50 ml of *water*. Add 0.2 ml of *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.00731 g of $C_6H_{10}O_4$.

Adrenaline

Epinephrine



$C_9H_{13}NO_3$ Mol. Wt. 183.2

Adrenaline is (*R*)-1-(3,4-dihydroxyphenyl)-2-methylaminoethanol.

Adrenaline contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_{13}NO_3$, calculated on the dried basis.

Category. Sympathomimetic.

Description. A white or creamy-white, microcrystalline powder or granules. It gradually darkens on exposure to light and air, decomposition being faster in the presence of moisture and at higher temperatures.

Identification

Test A may be omitted if tests B, C and D are carried out. Test C may be omitted if tests A, B and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *adrenaline IPRS* or with the reference spectrum of adrenaline.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum at 280 nm; absorbance at 280 nm, about 0.45.

C. To 1 ml of a neutral or faintly acid solution add dropwise a 0.25 per cent w/v solution of *ferric chloride* until an emerald-green colour is produced. Add *sodium bicarbonate solution* gradually; the solution changes first to blue and then to red.

D. To 1 ml of a 0.1 per cent w/v solution add 1 ml of a 1.0 per cent v/v solution of *2,5-diethoxytetrahydrofuran* in *glacial acetic acid*. Heat at 80° for 2 minutes, cool in ice and add 3 ml of a 2.0 per cent w/v solution of *4-dimethylaminobenzaldehyde* in a mixture of 19 volumes of *glacial acetic* acid and 1 volume of *hydrochloric acid*. Mix and allow to stand for 2 minutes. The solution becomes yellow and is similar to the one obtained by performing the test in the same manner but omitting the substance under examination (distinction from noradrenaline).

Tests

Specific optical rotation (2.4.22). −53.5° to −50.0°, determined in a freshly prepared 4.0 per cent w/v solution in *1 M hydrochloric acid*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions protected from light.

Solvent mixture A. Dissolve 5.0 g of *potassium dihydrogen phosphate* and 2.6 g of *sodium octanesulphonate* in *water* and dilute to 1000 ml with *water*, stir for at least 30 minutes, adjusted to pH 2.8 with *orthophosphoric acid.*

Solvent mixture B. 13 volumes of *acetonitrile* and 87 volumes of solvent mixture A.

Test solution. Dissolve 40 mg of the substance under examination in 5 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with solvent mixture B.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with solvent mixture B.

Reference solution (b). Dissolve 1.5 mg of *adrenaline impurity A IPRS (noradrenaline tartrate IPRS)* in solvent mixture B, add 1.0 ml of the test solution and dilute to 100.0 ml with solvent mixture B.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm),
- column temperature: 50°,
- mobile phase: A. a mixture of 5 volumes of *acetonitrile* and 95 volumes of solvent mixture A,
  B. a mixture of 45 volumes of *acetonitrile* and 55 volumes of solvent mixture A,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	92	8
15	50	50
20	92	8
25	92	8

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to adrenaline impurity A and adrenaline is not less than 3.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak at relative retention time of about 0.2, 0.8 and 1.3 with reference to the principal peak (retention time of Adrenaline is about 4 min) is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any peak at relative retention times of about 3.3 multiplied by a correction factor of 0.7 and about 3.7 multiplied by a correction factor of 0.6 with reference to the principal peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Phenones. Absorbance of a 0.2 per cent w/v solution in *0.1 M hydrochloric acid* at the maximum at about 310 nm (2.4.7), not more than 0.20, calculated on the dried basis.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 18 hours.

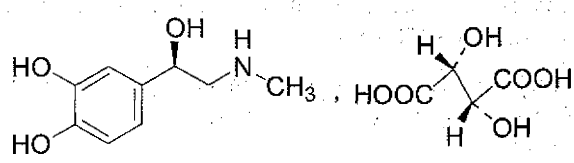
Assay. Dissolve 0.3 g in 50 ml of *anhydrous glacial acetic acid*, warming slightly, if necessary, to effect solution. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01832 g of $C_9H_{13}NO_3$.

Storage. Store protected from light in containers preferably filled with nitrogen.

Adrenaline Tartrate

Adrenaline Acid Tartrate; Adrenaline Bitartrate; Epinephrine Bitartrate.



$C_9H_{13}NO_3,C_4H_6O_6$ Mol. Wt. 333.3

Adrenaline tartrate is (*R*)-1-(3,4-dihydroxyphenyl)-2-methylaminoethanol hydrogen tartrate.

Adrenaline Tartrate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_9H_{13}NO_3,C_4H_6O_6$, calculated on the dried basis.

Category. Sympathomimetic.

Description. A white or greyish-white, crystalline powder. It darkens on exposure to air and light, decomposition being faster in the presence of moisture and at higher temperatures.

Identification

Dissolve 5 g in 50 ml of a 0.5 per cent w/v solution of *sodium metabisulphite* and make alkaline by addition of *ammonia*. Keep the mixture at room temperature for at least 15 minutes and filter. Reserve the filtrate for identification test C. Wash the precipitate with 3 quantities, each of 10 ml, of *methanol*. Evaporate to dryness at 80°. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *adrenaline IPRS* or with the reference spectrum of adrenaline.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum only at about 279 nm, about 0.45.

C. The filtrate reserved above gives the reaction (C) of tartrates (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution examined immediately after preparation is not more opalescent than opalescence standard OS2 (2.4.1) and not more intensely coloured than reference solution BYS4 (2.4.1).

pH (2.4.24). 2.8 to 4.0, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). −54.0° to −50.0°, determined in a freshly prepared 4.0 per cent w/v solution of residue obtained in identification test in *1 M hydrochloric acid*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions protected from light.

Solvent mixture A. Dissolve 5.0 g of *potassium dihydrogen phosphate* and 2.6 g of *sodium octanesulphonate* in *water* and dilute to 1000 ml with the same solvent, stir for at least 30 minutes, adjusted to pH 2.8 with *orthophosphoric acid*.

Solvent mixture B. 13 volumes of *acetonitrile* and 87 volumes of solvent mixture A.

Test solution. Dissolve 75 mg of the substance under examination in 5 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with solvent mixture B.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with solvent mixture B.

Reference solution (b). Dissolve 1.5 mg of *noradrenaline tartrate IPRS (adrenaline impurity A IPRS)* in solvent mixture B, add 1.0 ml of the test solution and dilute to 100.0 ml with solvent mixture B.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm),
- column temperature: 50°,
- mobile phase: A. a mixture of 5 volumes of *acetonitrile* and 95 volumes of solvent mixture A,
  B. a mixture of 45 volumes of *acetonitrile* and 55 volumes of solvent mixture A,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	92	8
15	50	50
20	92	8
25	92	8

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to adrenaline impurity A and adrenaline is not less than 3.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak at relative retention time of about 3.2 with reference to the principal peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The area of any peak at relative retention times of about 0.8 and 1.3 with reference to the principal peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Phenones. Absorbance of a 0.2 per cent w/v solution in *0.1 M hydrochloric acid* at the maximum at about 310 nm, not more than 0.10, calculated on the dried basis (2.4.7).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 18 hours.

Assay. Dissolve 0.3 g in 50 ml of *anhydrous glacial acetic acid*, warming slightly, if necessary, to effect solution. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03333 g of $C_9H_{13}NO_3,C_4H_6O_6$.

Storage. Store protected from light in containers preferably filled with nitrogen.

Adrenaline Injection

Adrenaline Bitartrate Injection; Adrenaline Acid Tartrate Injection; Adrenaline Tartrate Injection; Epinephrine Tartrate Injection

Adrenaline Injection is a sterile, isotonic solution containing 0.18 per cent w/v of Adrenaline Tartrate in Water for Injections.

Adrenaline Injection contains adrenaline tartrate equivalent not less than 90.0 per cent and not more than 115.0 per cent w/v of the stated amount of adrenaline, $C_9H_{13}NO_3$.

Usual strength. 0.1 per cent w/v.

Description. A clear, colourless or almost colourless solution.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponding to the peak in the chromatogram obtained with reference solution (a).

B. To 1 ml add dropwise a 0.25 per cent w/v solution of *ferric chloride* until an emerald-green colour is produced. Add *sodium bicarbonate solution* gradually; the solution changes first to blue and then to red.

C. To 10 ml add 2 ml of *disodium hydrogen phosphate solution* and sufficient *iodine solution* to produce a brown colour. Add *0.1 M sodium thiosulphate* dropwise until excess iodine is removed; a red colour is produced.

Tests

Appearance of solution. Examine the injection in a clear glass test-tube against a white background; it is not pinkish and does not contain a precipitate. If any yellow colour is observed, it is not more intense than a reference solution prepared by diluting 0.4 ml of *0.1 M iodine* to 100 ml with *water*, when viewed similarly.

pH (2.4.24). 2.8 to 3.6.

Noradrenaline. Determine by liquid chromatography (2.4.14).

Test solution. Use the undiluted injection.

Reference solution (a). A 0.0018 per cent w/v solution of *noradrenaline acid tartrate* in the mobile phase.

Reference solution (b). A solution containing 0.0018 per cent w/v of *noradrenaline-free adrenaline acid tartrate* and 0.0018 per cent w/v of *noradrenaline acid tartrate* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 to 10 µm) (Such as Nucleosil ODS),
- mobile phase: a buffer solution prepared by dissolving 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of *0.1 M disodium edetate* in 1000 ml of 5 per cent v/v solution of *methanol*, adjusted to pH 3.5 to 3.6 with *1 M sodium hydroxide*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 2.0.

Inject reference solution (a) and the test solution. The area of the peak corresponding to noradrenaline is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing 20 mg of Adrenaline Tartrate to 100.0 ml with the mobile phase.

Reference solution (a). A 0.02 per cent w/v solution of *adrenaline acid tartrate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.02 per cent w/v each of *adrenaline acid tartrate IPRS* and *noradrenaline acid tartrate* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a buffer solution prepared by dissolving 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate* and 2 ml of *0.1 M disodium edetate* to a mixture of 950 volumes of *water* and 50 volumes of *methanol*, adjusted to pH 3.5 with *1 M sodium hydroxide*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to adrenaline acid tartrate and noradrenaline acid tartrate is not less than 2.0.

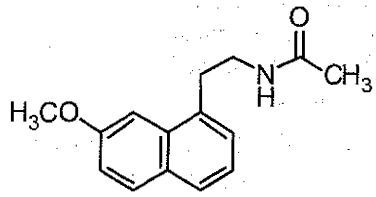
Inject reference solution (a) and the test solution.

Calculate the content of $C_9H_{13}NO_3$ in the injection.

Storage. Store protected from light, in a single dose or multiple dose container.

Labelling. The label states (1) the quantity of active ingredient in parts per 1000 or mg per ml in terms of equivalent amount of adrenaline; (2) that the injection should not be used if it is pinkish or darker than slightly yellow.

Agomelatine



$C_{15}H_{17}NO_2$ Mol Wt. 243.3

Agomelatine is *N*-[2-(7-methoxy-1-naphthyl)ethyl] acetamide.

Agomelatine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{17}NO$, calculated on the anhydrous basis.

Category. Antidepressant.

Description. A white to cream colour crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *agomelatine IPRS* or with the reference spectrum of agomelatine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile*.

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.02 per cent w/v solution of *agomelatine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- mobile phase: A. Dissolve 1.38 g of *sodium dihydrogen orthophosphate monohydrate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
50	30	70
52	80	20
60	80	20

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A 0.0025 per cent w/v solution of *agomelatine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1.38 g of *sodium dihydrogen orthophosphate monohydrate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

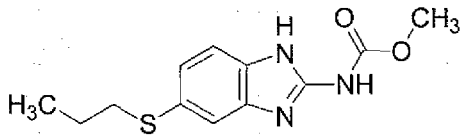
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{17}NO_2$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Albendazole



$C_{12}H_{15}N_3O_2S$ Mol. Wt. 265.3

Albendazole is methyl 5-propylthio-1*H*-benzimidazol-2-yl-carbamate.

Albendazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{15}N_3O_2S$, calculated on the dried basis.

Category. Anthelmintic.

Description. A white to pale buff-coloured powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *albendazole IPRS* or with the reference spectrum of albendazole.

B. In the test for Related substances, the principal peak in the chromatogram obtained with test solution corresponds to that of Albendazole in the chromatogram obtained with reference solution (b).

C. Melting point (2.4.21). 208° to 210°.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 1.0 per cent v/v solution of *sulphuric acid* in *methanol*.

Test solution. Dissolve 25 mg of the substance under examination in 5 ml of the solvent mixture and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dissolve 10 mg of the substance under examination in 10 ml of the solvent mixture and dilute to 100.0 ml with the mobile phase. Dilute 0.5 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b). Dissolve 50 mg each of the substance under examination and *oxibendazole IPRS* in 5 ml of the solvent mixture and dilute to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of 0.17 per cent w/v solution of *ammonium dihydrogen phosphate* and 70 volumes of *methanol*,
- flow rate: 0.7 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to albendazole and oxibendazole is not less than 3.0.

Inject reference solution (a) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent) and the sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Dissolve 0.5 g in 80 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02653 g of $C_{12}H_{15}N_3O_2S$.

Storage. Store protected from light.

Albendazole Oral Suspension

Albendazole Oral Suspension is a suspension of albendazole in a suitable vehicle.

Albendazole Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of albendazole $C_{12}H_{15}N_3O_2S$.

Usual strength. 200 mg per 5 ml.

Identification

A. To a volume of oral suspension containing 25 mg of Albendazole add 50 ml of *0.1 M sodium hydroxide* and shake with the aid of ultrasound for 10 minutes and dilute to 100 ml with *water* and filter. Dilute 1.0 ml of the solution to 10 ml with *water*. When examined in the range 240 nm to 340 nm (2.4.7),

the resulting solution exhibits a maximum at 308 nm, a minimum at 281 nm and a shoulder at 269 nm.

B. To a volume of oral suspension containing 25mg of Albendazole add 50 ml of *0.1 M hydrochloric acid* and shake with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with *0.1 M hydrochloric acid*, filter. Dilute 1.0 ml of the solution to 10.0 ml with *0.1 M hydrochloric acid*. When examined in the range 240 nm to 340 nm (2.4.7), the resulting solution exhibits a maximum at 292 nm, a minimum at 273 nm and a shoulder at 261 nm.

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 5.5.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE- Prepare the solutions immediately before use.

Solvent mixture. 30 volumes of *0.015 M ammonium dihydrogen orthophosphate* and 70 volumes of *methanol*.

Test solution. Dilute a quantity of the oral suspension containing 500 mg of Albendazole to 50.0 ml with 1.0 per cent w/v solution of *methanolic sulphuric acid*. Dilute 5.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dissolve 25 mg each of *albendazole IPRS* and *oxibendazole IPRS* in 5.0 ml of 1.0 per cent v/v solution of *methanolic sulphuric acid* and dilute to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *0.015 M ammonium dihydrogen orthophosphate*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 0.7 ml per minute,
- spectrophotometer set at 292 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
3	100	0
5	30	70
70	30	70
72	100	0
80	100	0

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 7.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the oral suspension containing 0.1 g of Albendazole, add 70 ml of 1 per cent v/v solution of *methanolic sulphuric acid*, mix with the aid of ultrasound for 1 minute and dilute to 100.0 ml with 1 per cent v/v *methanolic sulphuric acid*. Allow to stand and dilute 5 ml of the supernatant liquid to 25 ml with 1 per cent v/v *methanolic sulphuric acid*.

Reference solution. A 0.02 per cent w/v solution of *albendazole IPRS* in 1 per cent v/v solution of *methanolic sulphuric acid*.

Use chromatographic system as described under Related substances, operating the system for twice the retention time of albendazole peak.

Determine the weight per ml (2.4.29) and calculate the content of $C_{12}H_{15}N_3O_2S$.

Albendazole Tablets

Albendazole Tablets contain albendazole. The tablets may be chewable and may contain permitted flavouring and sweetening agents.

Albendazole Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of albendazole, $C_{12}H_{15}N_3O_2S$.

Usual strength. 400 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *chloroform*, 10 volumes of *ether* and 10 volumes of *glacial acetic acid*.

Test solution. Disperse a quantity of the powdered tablets containing 200 mg of Albendazole in 20 ml of a mixture of 18 volumes of *chloroform* and 1 volume of *formic acid*, warm the suspension on a water-bath for 15 minutes, cool and filter. Dilute 10 ml of the filtrate with an equal volume of *glacial acetic acid*.

Reference solution. A 0.5 per cent w/v solution of *albendazole IPRS* in *glacial acetic acid*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Extract a quantity of the powdered tablets containing 100 mg of Albendazole with 100 ml of *0.1 M methanolic hydrochloric acid*, filter and dilute 1.0 ml of the filtrate to 100 ml with *0.1 M sodium hydroxide*. The absorbance of the resulting solution at the maximum at about 309 nm (2.4.7) is about 0.74.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Dilute 1.0 ml of filtrate to 50.0 with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 308 nm (2.4.7). Calculate the content of $C_{12}H_{15}N_3O_2S$ in the medium taking 742 as the specific absorbance at 308 nm.

Q. Not less than 80 per cent of the stated amount of $C_{12}H_{15}N_3O_2S$.

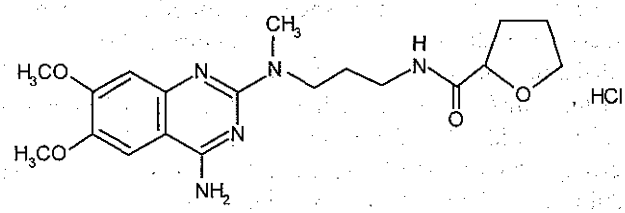
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of Albendazole, add 150 ml *0.1 M methanolic hydrochloric acid*, shake for 15 minutes and dilute to 250.0 ml with *0.1 M methanolic hydrochloric acid*. Mix, filter and dilute 5.0 ml of the filtrate to 250.0 ml with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 309 nm (2.4.7). Calculate the content of $C_{12}H_{15}N_3O_2S$ taking 742 as the specific absorbance at 309 nm.

Storage. Store protected from light.

Labelling. The label states, whereever applicable, the tablets should be chewed before swallowing.

Alfuzosin Hydrochloride



$C_{19}H_{27}N_5O_4,HCl$ Mol. Wt. 425.9

Alfuzosin Hydrochloride is (2*RS*)-*N*-[3-[(4-amino-6,7-dimethoxyquinazolin-2-yl)methylamino]propyl]tetrahydrofuran-2-carboxamide hydrochloride.

Alfuzosin Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{19}H_{27}N_5O_4,HCl$, calculated on the anhydrous basis.

Category. Indicated in benign prostatic hyperplasia.

Description. A white or almost white, crystalline powder, slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *alfuzosin hydrochloride IPRS* or with the reference spectrum of alfuzosin hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 5.5, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1 volume of *tetrahydrofuran*, 20 volumes of *acetonitrile* and 80 volumes of a solution prepared by dissolving 5.0 ml of *perchloric acid* in 900 ml of *water*, adjusted to pH 3.5 with *dilute sodium hydroxide solution* and dilute to 1000 ml with *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Name	Relative retention time
Alfuzosin impurity D[1]	0.4
Alfuzosin hydrochloride (Retention time: about 8 minutes)	1.0
Alfuzosin impurity A[2]	1.2

[1]*N*-(4-amino-6,7-dimethoxyquinazolin-2-yl)-*N*-methylpropane-1,3-diamine,
[2]*N*-[3-[(4-amino-6,7-dimethoxyquinazolin-2-yl)methylamino]propyl] furan-2-carboxamide.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to alfuzosin impurity D is not more than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). The area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Weigh 0.3 g, dissolve in 40 ml of *acetic acid*, add 40 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*. Determine the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04259 g of $C_{19}H_{27}N_5O_4,HCl$.

Storage. Store protected from light and moisture.

Alfuzosin Prolonged-release Tablets

Alfuzosin Hydrochloride Prolonged-release Tablets

Alfuzosin Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Alfuzosin Prolonged-release Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of alfuzosin hydrochloride, $C_{19}H_{27}N_5O_4,HCl$.

Usual strength. 10 mg.

Identification

Disperse a quantity of the powdered tablets containing 30 mg of Alfuzosin Hydrochloride with 150 ml of *water* for 5 minutes and filter. To the filtrate, add 10 ml of *18 M ammonia*, extract with two 25 ml quantities of *dichloromethane*, wash the combined extracts with 10 ml of *water*, dry over *sodium sulphate* and evaporate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *alfuzosin hydrochloride IPRS* treated in the same manner or with the reference spectrum of alfuzosin.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 15 mg of Alfuzosin Hydrochloride in 70 ml of *methanol* with the aid of ultrasound for 30 minutes, add 10 ml of *0.01M hydrochloric acid* , cool, dilute to 100.0 ml with *methanol* and filter. Dilute 1.0 ml of the solution to 5.0 ml with the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: mobile phase: a mixture of 1 volume of *tetrahydrofuran*, 20 volumes of *acetonitrile* and 80 volumes of sodium perchlorate solution prepared by dissolving 5 ml of *perchloric acid* to 900 ml of *water*, adjusted to pH 3.5 with *2 M sodium hydroxide* and dilute to 1000 ml with *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent), the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than

0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets using following test solution.

Test solution. Disperse 1 tablet in 70 ml of *methanol* with the aid of ultrasound for 30 minutes, add 10 ml of *0.01M hydrochloric acid*, cool, dilute to 100.0 ml with *methanol* and filter. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 10 mg of Alfuzosin Hydrochloride in 70 ml of *methanol* with the aid of ultrasound for 30 minutes, add 10 ml of *0.01M hydrochloric acid*, cool, dilute to 100 ml with *methanol* and filter. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution. A 0.001 per cent w/v solution of *alfuzosin hydrochloride IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{27}N_5O_4$,HCl in the tablets.

Alfuzosin Tablets

Alfuzosin Hydrochloride Tablets

Alfuzosin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of alfuzosin hydrochloride, $C_{19}H_{27}N_5O_4$,HCl.

Usual strength. 10 mg.

Identification

Disperse a quantity of the powdered tablets containing 30 mg of Alfuzosin Hydrochloride with 150 ml of *water* for 5 minutes and filter. To the filtrate, add 10 ml of *18 M ammonia*, extract with two 25-ml quantities of *dichloromethane*, wash the combined extracts with 10 ml of *water*, dry over *sodium sulphate* and evaporate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *alfuzosin hydrochloride IPRS* treated in the same manner or with the reference spectrum of alfuzosin.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the mobile phase to obtain a solution containing a 0.0001 per cent w/v of Alfuzosin Hydrochloride.

Reference solution. A 0.0001 per w/v solution of *alfuzosin hydrochloride IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

- injection volume: 100 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{27}N_5O_4$,HCl in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{19}H_{27}N_5O_4$,HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 15 mg of Alfuzosin Hydrochloride in 70 ml of *methanol* with the aid of ultrasound for 30 minutes, add 10 ml of *0.01M hydrochloric acid*, cool, dilute to 100.0 ml with *methanol* and filter. Dilute 1.0 ml of the solution to 5.0 ml with the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1 volume of *tetrahydrofuran*, 20 volumes of *acetonitrile* and 80 volumes of sodium perchlorate solution prepared by dissolving 5 ml of *perchloric acid* to 900 ml of *water*, adjusted to pH 3.5 with *2 M sodium hydroxide* and add sufficient *water* to produce 1000 ml,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any

secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent), the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets using following test solution.

Test solution. Disperse 1 tablet in 70 ml of *methanol*, with the aid of ultrasound for 30 minutes, add 10 ml of *0.01M hydrochloric acid*, cool, dilute to 100.0 ml with *methanol* and filter. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 10 mg of Alfuzosin Hydrochloride in 70 ml of *methanol* with the aid of ultrasound for 30 minutes, add 10 ml of *0.01M hydrochloric acid*, cool, dilute to 100.0 ml with *methanol* and filter. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution. A 0.001 per cent w/v solution of *alfuzosin hydrochloride IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{27}N_5O_4$,HCl in the tablets.

Alginic Acid

Polymannuronic Acid

Alginic acid is a hydrophilic colloidal mixture of polyuronic acids, $[(C_6H_8O_6)_n]$, composed of residues of D-mannuronic acid and L-guluronic acid extracted with dilute alkali from various species of brown seaweeds (Fam. Phaeophyceae).

Alginic Acid contains not less than 19.0 per cent and not more than 25.0 per cent of carboxylic acid groups (COOH), calculated on the dried basis.

Category. Pharmaceutical aid.

Description. A white to yellowish-white, fibrous powder.

Identification

A. To 5 ml of a 0.75 per cent w/v solution in *0.1 M sodium hydroxide* add 1 ml of *calcium chloride solution*; a gelatinous precipitate is formed.

B. To 5 ml of the solution obtained in test A add 1 ml of *2 M sulphuric acid*; a gelatinous precipitate is formed.

C. To about 5 mg in a test-tube add 5 ml of *water*, 1 ml of a freshly prepared 1 per cent w/v solution of *1,3-naphthalenediol* in *ethanol (95 per cent)* and 5 ml of *hydrochloric acid*. Heat the mixture to boiling, boil gently for 3 minutes and cool to about 15°. Transfer the contents of the test-tube to a small separator with the aid of 5 ml of *water* and extract with 15 ml of *di-isopropyl ether*; the di-isopropyl ether extract exhibits a deep purple colour which is more intense than that exhibited by a blank prepared in the same manner without the substance under examination.

Tests

pH (2.4.24). 1.5 to 3.5, determined in a 3.0 per cent w/v dispersion in *water*.

Acid value. Not less than 230, calculated on the dried basis and determined in the following manner. Weigh 1.0 g and suspend in a mixture of 50 ml of *water* and 30 ml of a 4.4 per cent w/v solution of *calcium acetate*. Shake vigorously, allow the mixture to stand for 1 hour, add *phenolphthalein solution* and titrate the liberated acetic acid with *0.1 M sodium hydroxide*. Carry out a blank titration.

Calculate the acid value from the expression 5.611 *A/W*, where *A* is the volume, in ml, of *0.1 M sodium hydroxide* consumed and W is the weight, in g, of the sample.

Arsenic (2.3.10). Mix 2.0 g with 5 ml of *sulphuric acid*, add a few glass beads and digest at a temperature not exceeding 120° until charring begins. Additional sulphuric acid may be added if necessary but the total volume of acid added should not exceed 10 ml. Add cautiously, dropwise, *hydrogen peroxide solution (100 vol)* allowing the reaction to subside and again heating between addition of drops. Discontinue heating if foaming becomes excessive. When the reaction has abated, heat cautiously rotating the flask occasionally. Maintain oxidising conditions at all times during the digestion by adding small quantities of the hydrogen peroxide solution whenever the mixture turns brown or darkens. Continue the digestion until the organic matter has been destroyed, gradually raising the temperature until fumes of sulphur trioxide are copiously evolved and the solution becomes colourless or has only a light straw colour. Cool, add cautiously 10 ml of *water*, mix, and again evaporate till there is strong fuming, repeating this procedure to remove any trace of hydrogen peroxide. Cool, add cautiously 10 ml of *water*, wash the sides of the flask with

a few ml of *water* and dilute with *water* to 35 ml. The resulting solution complies with the limit test for arsenic (5 ppm).

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm). Use *nitric acid* in place of *sulphuric acid* to wet the sample.

Microbial contamination (2.2.9). 1 g is free from *Escherichia coli* and 10 g is free from *Salmonella* and *Shigella*.

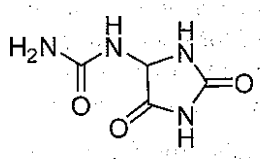
Total ash (2.3.19). Not more than 4.0 per cent, determined on 0.5 g by Method B.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 0.1 g by drying in an oven at 105° for 4 hours.

Assay. Weigh 0.25 g, add 25 ml of *water* and 25.0 ml of *0.1 M sodium hydroxide* and titrate with *0.1 M hydrochloric acid* using 0.2 ml of *dilute phenolphthalein solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.004502 g of carboxylic acid groups (COOH).

Allantoin



$C_4H_6N_4O_3$ Mol. Wt. 158.1

Allantoin is (*RS*)-(2,5-dioxo-4-imidazolidinyl)urea.

Allantoin contains not less than 98.5 per cent and not more than 101.0 per cent of $C_4H_6N_4O_3$, calculated on the dried basis.

Category. Astringent.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *allantoin IPRS* or with the reference spectrum of allantoin.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Boil 20 mg with a mixture of 1 ml each of *dilute sodium hydroxide solution* and *water*, allow to cool. Add 1 ml of *dilute hydrochloric acid*. To 0.1 ml of the solution add 0.1 ml of a 10 per cent w/v solution of *potassium bromide*, 0.1 ml of a 2 per cent w/v solution of *resorcinol* and 3 ml of *sulphuric acid*. Heat for 10 minutes on a water bath; a dark blue colour develops, which becomes red after cooling and pouring into about 10 ml of *water*.

D. Heat about 0.5 g, ammonia vapour is evolved, which turns *red litmus paper* blue.

Tests

Acidity or alkalinity. To 5 ml of a 0.5 per cent w/v solution in *carbon dioxide-free water* with heating if necessary (solution A), add 5 ml of *carbon dioxide-free water*, 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*, the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*, the solution is red.

Optical rotation (2.4.22). −0.10° to +0.10°, determined on solution A.

Reducing substances. Shake 1.0 g with 10 ml of *water* for 2 minutes, filter. Add 1.5 ml of *0.02 M potassium permanganate*. The solution must remain violet for at least 10 minutes.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose*.

Mobile phase. A mixture of 15 volumes of *glacial acetic acid*, 25 volumes of *water* and 60 volumes of *butanol*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 5.0 ml of *water* with heating and allow to cool, dilute to 10.0 ml with *methanol*. (*Use the solution immediately after preparation*).

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with a mixture of 1 volume of *methanol* and 1 volume of *water*.

Reference solution (a). A 0.1 per cent w/v solution of *allantoin IPRS* in a mixture of 1 volume of *methanol* and 1 volume of *water*.

Reference solution (b). Dissolve 10 mg of *urea* in 10.0 ml of *water*. Dilute 1.0 ml of the solution to 10.0 ml with *methanol*.

Reference solution (c). Mix 1.0 ml each of reference solution (a) and reference solution (b).

Apply to the plate 10 µl of test solution (a) and 5 µl each of test solution (b), reference solution (a), (b) and (c). Allow the mobile phase to rise 10 cm. Dry the plate in air, and spray with a 0.5 per cent w/v solution of *dimethylaminobenzaldehyde* in a mixture of 1 volume of *hydrochloric acid* and 3 volumes of *methanol*. Dry the plate in a current of hot air. Examine in daylight after 30 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid

unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

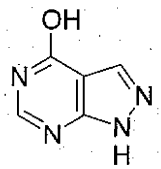
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.1 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 120 mg in 40 ml of *water*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01581 g of $C_4H_6N_4O_3$.

Allopurinol



$C_5H_4N_4O$ Mol. Wt. 136.1

Allopurinol is a tautomeric mixture of 1*H*-pyrazolo[3,4-*d*] pyrimidin-4-ol and 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one.

Allopurinol contains not less than 98.0 per cent and not more than 101.0 per cent of $C_5H_4N_4O$, calculated on the dried basis.

Category. Uricosuric agent.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *allopurinol IPRS* or with the reference spectrum of allopurinol.

B. Dissolve 0.1 g in 10 ml of *0.1 M sodium hydroxide* and add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml; dilute 10.0 ml to 100.0 ml with *0.1 M hydrochloric acid* and dilute 10.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*. When examined in the range 220 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 250 nm and a minimum at about 231 nm; ratio of the absorbance at the minimum at about 231 nm to that at the maximum at about 250 nm, 0.52 to 0.62.

C. Dissolve 50 mg in 5 ml of *dilute sodium hydroxide solution*, add 1 ml of *alkaline potassium mercuri-iodide solution*, heat to boiling and allow to stand; a flocculent yellow precipitate is produced.

D. Shake about 0.1 g with 5 ml of *dilute sodium hydroxide solution*, add 3 ml of *lithium and sodium molybdophosphotungstate solution* and 5 ml of a 20 per cent w/v solution of *sodium carbonate*; a grey-blue colour is produced.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *2 M sodium hydroxide* is clear, (2.4.1) and not more intensely coloured than reference solution YS6 or GYS4 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions, store and inject at 8°, using a cooled autosampler.

Test solution. Dissolve 25 mg of the substance under examination in 2.5 ml of a 0.4 per cent w/v solution of *sodium hydroxide* and dilute immediately to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). A solution containing 5.0 mg each of *allopurinol impurity A IPRS (5-amino-1H-pyrazole-4-carboxamide IPRS), allopurinol impurity B IPRS (5-(formylamino)-1H-pyrazole-4-carboxamide IPRS)* and *allopurinol impurity C IPRS (5-(4H-1,2,4–triazol-4-yl)-1H-pyrazole-4-carboxamide IPRS)* in 5.0 ml of a 0.4 per cent w/v solution of *sodium hydroxide* and dilute immediately to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a 0.125 per cent w/v solution of *potassium dihydrogen phosphate*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

The elution order of the peaks is allopurinol impurity A, allopurinol impurity B, allopurinol impurity C and allopurinol. The retention time for allopurinol is about 10 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to allopurinol impurity B and allopurinol impurity C is not less than 1.1.

Inject reference solution (a), (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to allopurinol impurity A is not more than

twice the area of corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of the peak due to allopurinol impurity C is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of the peak due to allopurinol impurity B and of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks other than allopurinol impurity A, B and C is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.2 g and dissolve with gentle heating, if necessary, in 50 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01361 g of $C_5H_4N_4O$.

Allopurinol Tablets

Allopurinol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of allopurinol, $C_5H_4N_4O$.

Usual strengths. 100 mg; 300 mg.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 250 nm.

B. Disperse a quantity of the powdered tablets containing 0.1 g of Allopurinol with 5 ml of *dilute sodium hydroxide solution*, add 3 ml of *lithium and sodium molybdo-phosphotungstate solution* and 5 ml of a 20 per cent w/v solution of *sodium carbonate*; a grey-blue colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium: 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 75 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7). Calculate the content of allopurinol, $C_5H_4N_4O$ in the medium from the absorbance obtained from a solution containing 0.001 per cent w/v of *allopurinol IPRS* prepared by dissolving in minimum amount of *0.1 M sodium hydroxide* and diluted with the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_5H_4N_4O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.1 g of Allopurinol with 10 ml of *0.1 M sodium hydroxide* with the aid of ultrasound and immediately dilute to 200.0 ml with mobile phase A, filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase A. Further dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution (b). Dissolve 10 mg of *allopurinol impurity A IPRS (5–amino–1H–pyrazole–4–carboxamide IPRS)* in mobile phase A, add 20 ml of the test solution and immediately dilute to 100.0 ml with mobile phase A. Further dilute 1.0 ml of the solution to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: A. a mixture of 10 volumes of *methanol* and 90 volumes of a 0.125 per cent w/v solution of *potassium dihydrogen orthophosphate*,
  B. a mixture of 30 volumes of *methanol* and 70 volumes of a 0.125 per cent w/v solution of *potassium dihydrogen orthophosphate*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
30	0	100
40	0	100
42	100	0

The elution order of the peaks is allopurinol impurity A, allopurinol impurity B, allopurinol impurity C and allopurinol. The retention time for allopurinol is about 10 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to allopurinol impurity A and allopurinol is not less than 3.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to allopurinol impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of unresolved double peak, the peak at retention time of about 6.1 minutes is not more than twice the area of the principle peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of the areas of any other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of Allopurinol with 20 ml of *0.05 M sodium hydroxide* for 15 to 20 minutes, add 75 ml of *0.1 M hydrochloric acid* shake for 10 minutes, add sufficient *0.1 M hydrochloric acid* to produce 250.0 ml, filter. Dilute 5.0 ml of the filtrate to 250.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7) using *0.1 M hydrochloric acid* as the blank.

Calculate the content of $C_5H_4N_4O$, taking 563 as the specific absorbance at 250 nm.

Aloes

Aloes is the dried juice of the leaves of *Aloe barbadensis* Miller *(A. vera* Linn), known in commerce as Curacao Aloes or Barbados Aloes, or of *A. ferox* Miller and hybrids of this species with *A. africana* Miller and *A. spicata* Baker, known in commerce as Cape Aloes (Fam. Liliaceae). Indian Aloes of commerce is obtained from *A. barbadensis*.

Aloes contains not less than 50.0 per cent of water-soluble extractive. Curacao Aloes contains not less than 18.0 per cent and Cape Aloes not less than 28.0 per cent of hydroxyanthracene derivatives, calculated as anhydrous barbaloin.

Category. Laxative.

Description. *Unground Curacao Aloes* — Brownish-black, opaque masses; fractured surface uneven, waxy and somewhat resinous.

Unground Cape Aloes — Dark-brown or greenish-brown to olive-brown masses; fractured surface shiny and conchoidal.

Identification

Mix 0.5 g with 50 ml of *water*, boil until nearly dissolved, cool, add 0.5 g of *silica gel* and filter. On the filtrate carry out the following tests.

A. Heat 5 ml with 0.2 g of *borax* until dissolved, add a few drops of the solution to a test-tube nearly filled with *water*; a green fluorescence is produced.

B. Mix 2 ml with 2 ml of *bromine water*; a pale yellow precipitate is produced. The supernatant liquid is violet with Curacao Aloes; no such violet colour appears with Cape Aloes.

C. Mix 5 ml with 2 ml of *nitric acid*; with Cape Aloes a reddish-yellow colour is produced; with Socotrine Aloes a pale brownish-yellow colour is produced; with Cape Aloes a yellowish-brown colour passing rapidly to green is produced.

D. Determine by thin-layer chromatography (2.4.17), coating the plate with silica *gel G*.

Mobile phase. A mixture of 100 volumes of *ethyl acetate*, 17 volumes of *methanol* and 13 volumes of *water*.

Test solution. Heat 0.5 g, in powder, with 20 ml *methanol* to boiling on a water-bath, shake well, decant the supernatant liquid, keep at 4° and use within 24 hours.

Reference solution. Dissolve 50 mg of *barbaloin* in 10.0 ml *methanol*.

Apply to the plate 5 µl of each solution as bands 20 mm x 3 mm. Allow the mobile phase to rise 15 cm. Dry the plate in a current of air, spray with a 10 per cent w/v solution of *potassium hydroxide* in *methanol* and examine under ultraviolet light at 365 nm. The chromatogram obtained with the reference solution shows a yellow band with an R_f value of 0.4 to 0.5. In the case of Curacao Aloes, the chromatogram obtained with the test solution shows a yellow fluorescent band corresponding to that due to barbaloin in the chromatogram obtained with the reference solution and in the lower part a light blue fluorescent band (corresponding to aloesine). In the case of Cape Aloes, the test solution shows a yellow fluorescent band corresponding to that due to barbaloin in the chromatogram obtained with the reference solution and in the lower part two yellow fluorescent bands (due to aloinosides A and B) as well as a blue fluorescent band (due to aloesine). Heat the plate at 110° for 5 minutes. In the case of Curacao Aloes, with the test solution a violet fluorescent band appears just below the yellow band corresponding to barbaloin while in the case of Cape Aloes no such violet band appears.

Tests

Ethanol-insoluble substances. Weigh 1.0 g, in fine powder, and add to 50 ml of *ethanol (95 per cent)* in a flask. Reflux the mixture for 15 minutes. Remove the source of heat and set aside for 1 hour, shaking frequently, filter through a small dried and tared filter paper or suitable filtering crucible and wash the residue on the filter with *ethanol (95 per cent)* till the washings are colourless. The residue after drying to constant weight at 105° weighs not more than 0.1 g.

Water-soluble extractive. Weigh 2.0 g, in fine powder, and macerate with about 60 to 70 ml of *water* in a flask. Shake the mixture at 30-minute intervals for 8 hours and allow to stand for a further 16 hours without shaking. Filter, wash the flask and the residue with small portions of *water*, passing the washings through the filter until the filtrate measures 100 ml. Evaporate 50 ml of this filtrate to dryness in a tared dish on a water-bath and dry at 105° for 3 hours; the residue weighs not less than 0.5 g.

Total ash (2.3.19). Not more than 0.5 per cent, determined on 1.0 g by Method A.

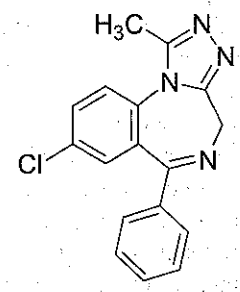
Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Moisten 0.2 g, in fine powder, with 2 ml of *methanol*, add 5 ml of *water* at about 60°, mix, add a further 75 ml of *water* at about 60°, shake for 30 minutes, cool, filter through a filter paper, washing the flask with 20 ml of *water* and add sufficient *water* to the combined filtrate and washings to produce 1000.0 ml. Transfer 10.0 ml of the solution to a flask containing 1 ml of a 60 per cent w/v solution of *ferric chloride hexahydrate* and 6 ml of *hydrochloric acid*, heat in a water-bath under a reflux condenser for 4 hours so that the water level is always above that of the liquid in the flask, cool, transfer the solution to a separating funnel, rinsing the flask successively with 4 ml of *1 M sodium hydroxide* and 4 ml of *water* and adding the rinsings to the contents of the separating funnel. Extract with three quantities, each of 20 ml, of *carbon tetrachloride* and wash the combined carbon tetrachloride layers with two quantities, each of 100 ml, of *water*, discarding the washings. Dilute the organic phase to 100.0 ml with *carbon tetrachloride*, evaporate 20.0 ml carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of *1 M sodium hydroxide*. Immediately measure the absorbance of the resulting solution at the maximum at about 440 nm and at about 500 nm (2.4.7) Calculate the content of anhydrous barbaloin, taking 200 as the specific absorbance at 500 nm. The result of the Assay is not valid unless the ratio of the absorbance at about 500 nm to that at about 440 nm is not less than 1.9.

Storage. Store protected from light and moisture.

Labelling. The label states whether the material is Curacao Aloes or Cape Aloes.

Alprazolam



$C_{17}H_{13}ClN_4$ Mol. Wt. 308.8

Alprazolam is 8-chloro-1-methyl-6-phenyl-4*H*-1,2,4-triazolo[4,3-*a*][1,4]benzodiazepine.

Alprazolam contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{13}ClN_4$, calculated on the dried basis.

Category. Anxiolytic.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *alprazolam IPRS* or with the reference spectrum of alprazolam.

B. Dissolve 10 mg in *methanol* and dilute to 500.0 ml with *methanol*. Dilute 20.0 ml of the solution to 100.0 ml with *methanol*. When examined in the range 210 nm to 360 nm (2.4.7), the solution shows an absorption maximum at about 220 nm.

C. Melts at about 225° (2.4.21).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in *dimethylformamide* and dilute 10.0 ml with *dimethylformamide*.

Reference solution. Dilute 5.0 ml of the test solution to 100.0 ml with *dimethylformamide*. Dilute 0.5 ml of the solution to 10.0 ml with *dimethyformamide*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with phenylsilane bonded to porous silica (5 µm),
- column temperature: 40°,

– mobile phase: A. a mixture of 44 volumes of a buffer solution prepared by dissolving 7.7 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 4.2 with *glacial acetic acid* and 56 volumes of *methanol*,
B. a mixture of 5 volumes of a buffer solution prepared by dissolving 7.7 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 4.2 with *glacial acetic acid* and 95 volumes of *methanol*,
– a gradient programme using the conditions given below,
– flow rate: 2 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
15	98	2
35	1	99
40	1	99
42	98	2

Equilibrate the column for at least 30 minutes with the initial eluent composition. For subsequent chromatographs equilibrate the column for 10 minutes with the same eluent. The retention time of the principal peak is about 10 minutes.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.25 per cent). Ignore any peaks with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in *acetonitrile* and dilute to 100.0 ml with *acetonitrile*. Dilute 10.0 ml of the solution to 100.0 ml with *acetonitrile*.

Reference solution. A 0.0025 per cent w/v solution of *alprazolam IPRS* in *acetonitrile*.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (3 to 10 µm),
– mobile phase: a mixture of 850 volumes of *acetonitrile*, 80 volumes of *chloroform*, 50 volumes of *1-butanol*, 20 volumes of *water* and 0.5 volume of *glacial acetic acid*,
– flow rate: 2 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 10 µl or 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{13}ClN_4$.

Storage. Store protected from light.

Alprazolam Prolonged-release Tablets

Alprazolam Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Alprazolam Prolonged-release tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of alprazolam, $C_{17}H_{13}ClN_4$.

Usual strengths. 0.5 mg; 1 mg; 1.5 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

Test solution. Disperse 1 tablet in *acetonitrile* with the aid of ultrasound. Add about 25 ml of *water*, sonicate for 15 minutes, add about 12 ml of *acetonitrile* and further sonicate for 15 minutes, dilute to 50.0 ml with the mobile phase, filter.

Reference solution. A 0.05 per cent w/v solution of *alprazolam IPRS* in *acetonitrile*. Dilute if necessary with the mobile phase to obtain a concentration similar to that of the test solution.

Calculate the content of $C_{17}H_{13}ClN_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 2.5 mg of Alprazolam in 1.0 ml of *acetonitrile* and 50 ml of *water* with the aid of ultrasound for 15 minutes with occasional shaking. Further add 35 ml of *acetonitrile* and sonicate for 20 minutes and dilute to 100.0 ml with *acetonitrile*. Centrifuge at 3500 rpm for 20 minutes. Dilute the clear supernatant liquid with the mobile phase to obtain a solution containing 0.00125 per cent w/v of alprazolam.

Reference solution. A 0.05 per cent w/v solution of *alprazolam IPRS* in *acetonitrile*. To 5.0 ml of the solution, add 50 ml of *water* and dilute to 100.0 ml with *acetonitrile*. Further dilute the solution with the mobile phase to obtain a solution containing 0.00125 per cent w/v of alprazolam.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 0.8 g of *monobasic potassium phosphate* and 0.2 g of *dibasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 6.0 with *dilute orthophosphoric acid* or *potassium hydroxide*, 35 volumes of *acetonitrile* and 5 volumes of *tetrahydrofuran*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{13}ClN_4$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Alprazolam Tablets

Alprazolam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of alprazolam, $C_{17}H_{13}ClN_4$.

Usual strengths. 0.25 mg; 0.5 mg; 1 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 500 ml of a buffer solution prepared by dissolving 0.8 g of *monobasic potassium phosphate* and 0.2 g of *dibasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 6.0 with *orthophosphoric acid*,

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. A 0.005 per cent w/v solution of *alprazolam IPRS* in *methanol*. Dilute the solution with the dissolution medium to obtain a solution of about the same concentration as the test solution.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of buffer solution, 35 volumes of *acetonitrile* and 5 volumes of *tetrahydrofuran*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 500 theoretical plates, and the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{13}ClN_4$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{13}ClN_4$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Transfer one tablet to a container, add 0.4 ml of *water* on to the tablet, allow the tablet to stand for 2 minutes and swirl the container to disperse the tablet. Add sufficient *acetonitrile* to produce a solution containing 0.0025 per cent w/v of alprazolam. Shake to mix and centrifuge, if necessary.

Reference solution. A 0.0025 per cent w/v solution of *alprazolam IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (5 to 10 µm),
- mobile phase: a mixture of 850 volumes of *acetonitrile*, 80 volumes of *chloroform*, 50 volumes of *1-butanol*, 20 volumes of *water* and 0.5 volume of *glacial acetic acid*.
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl or 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{13}ClN_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Place 5 tablets in a flask, add 2 ml of *water* and swirl to disperse the tablets. Add sufficient *acetonitrile* to produce 25.0 ml. Shake for 10 to 15 minutes and centrifuge if necessary. Dilute a portion of the clear solution with *acetonitrile* to produce a solution containing 0.0025 per cent w/v of alprazolam.

Reference solution. A 0.0025 per cent w/v solution of *alprazolam IPRS* in *acetonitrile*.

Use chromatographic system as described under Uniformity of content.

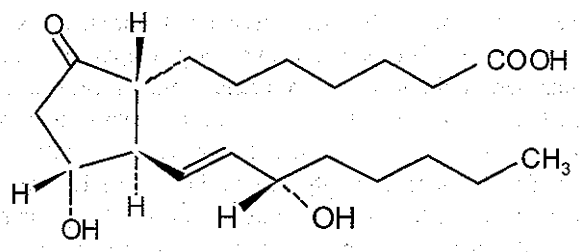
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{13}ClN_4$ in the tablets.

Storage. Store protected from light.

Alprostadil



$C_{20}H_{34}O_5$ Mol. Wt. 354.5

Alprostadil is (11α,13*E*,15*S*)-11,15-dihydroxy-9-oxo-prost-13-en-1-oic acid.

Alrostadil contains not less than 95.0 per cent and not more than 102.5 per cent of $C_{20}H_{34}O_5$, calculated on the anhydrous basis.

Category. Indicated in erectile dysfunction.

Description. A white or slightly yellowish, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *alprostadil IPRS* or with the reference spectrum of alprostadil.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.3.22). – 70.0° to – 60.0°, determined on 0.5 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Prepare the solutions protected from light.

Buffer solution. Dissolve 3.9 g of *sodium dihydrogen phosphate* in *water* and dilute to 1000.0 ml with *water*, adjusted to pH 2.5 with 0.3 per cent v/v solution of *orthophosphoric acid*.

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). Dilute 100 µl of the test solution to 20.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.005 per cent w/v each of *alprostadil impurity H IPRS* and *alprostadil IPRS* in the solvent mixture.

Reference solution (c). In order to prepare in situ the degradation compounds (alprostadil impurity A and alprostadil impurity B), dissolve1 mg of the substance under examination in100 µl of *1 M sodium hydroxide* (the solution becomes brownish-red), wait for 3 minutes and add 100 µl of *1 M orthophosphoric acid* (yellowish-white opalescent solution); dilute to 5.0 ml with the solvent mixture.

System A

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with base deactivated octylsilane bonded to porous silica (4 µm),
- column temperature: 35°,
- mobile phase: A. a mixture of 74 volumes of the buffer solution and 26 volumes of *acetonitrile*,
  B. a mixture of 20 volumes of the buffer solution and 80 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
75	100	0
76	0	100
86	0	100
87	100	0
102	100	0

Name	Relative retention time	Correction factor
Alprostadil impurity G[1]	0.8	0.7
Alprostadil impurity F[2]	0.88	0.8
Alprostadil impurity D[3]	0.90	1.0
Alprostadil impurity H[4]	0.96	0.7
Alprostadil (retention time: about 63 minutes)	1.0	---
Alprostadil impurity E[5]	1.1	0.7

[1]dinoprostone,

[2]8-epiprostaglandin E_1,

[3]15-epiprostaglandin E_1,

[4](5E)-prostaglandin E_2,

[5]11-epiprostaglandin E_1.

Inject reference solution (b). The test is not valid unless the between the peaks due to alprostadil impurity H and alprostadil is not less than 1.5.

System B

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with base deactivated octylsilane bonded to porous silica (4 µm),
- column temperature: 35°,
- mobile phase: A. a mixture of 60 volumes of the buffer solution and 40 volumes of *acetonitrile,*
  B. a mixture of 20 volumes of the buffer solution and 80 volumes of *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
50	100	0
51	0	100
61	0	100
62	100	0
72	100	0

Name	Relative retention time	Correction factor
Alprostadil (retention time: about 7 minutes)	1.0	—
Alprostadil impurity C[6]	1.36	1.9
Alprostadil impurity K[7]	1.85	0.06
Alprostadil impurity A[8]	2.32	0.7
Alprostadil impurity B[9]	2.45	1.5
Alprostadil impurity I[10]	4.00	1.0
Alprostadil impurity J[11]	5.89	1.0

[6]15-oxoprostaglandin E_1,

[7]triphenylphosphine oxide,

[8]prostaglandin A_1,

[9]prostaglandin B_1,

[10]prostaglandin E_1,ethyl ester,

[11]prostaglandin E_1,isopropyl ester.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to alprostadil impurity A and alprostadil impurity B is not less than 1.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to alprostadil impurity A is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent), the area of any peak corresponding to alprostadil impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of any other secondary peak is not more than 1.8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.9 per cent) and not more than 1 such peak has an area more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (2.3.43). Not more than 0.5 per cent, determined on 0.05 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances, System A.

NOTE— Prepare the solutions protected from light.

Solvent mixture. Equal volumes of *acetonitrile* and *water.*

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 25.0 ml with the solvent mixture. Dilute 3.0 ml of the solution to 20.0 ml with the solvent mixture.

Reference solution. A 0.006 per cent w/v solution of *alprostadil IPRS* in the solvent mixture.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{34}O_5$.

Storage. Store at a temperature of 2° to 8°.

Alprostadil Injection

Alprostadil Injection is a sterile solution of Alprostadil in Dehydrated Alcohol.

Alprostadil Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of alprostadil, $C_{20}H_{34}O_5$.

Usual strength. 500 µg per ml for intravascular or intramuscular use.

Identification

Dry an amount of injection containing 2 mg of alprostadil on 500 mg of *potassium bromide* at about 40° to 50° under vacuum. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *alprostadil IPRS* or with the reference spectrum of alprostadil.

Tests

Water (2.3.43). Not more than 0.4 per cent.

Bacterial endotoxins (2.2.3). Not more than 5 Endotoxin Units per 100 µg of Alprostadil.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Gently evaporate a volume of injection containing 0.25 mg of Alprostadil to dryness using a stream of nitrogen. Add 150 µl of freshly prepared 4.0 per cent w/v solution of *α-bromo-2'-acetonaphthone* in *acetonitrile*. Add 150 µl of a freshly prepared 0.5 per cent w/v solution of *diisopropylethylamine* in *acetonitrile* to the container, cap and dissolve with the aid of ultrasound. Heat the container at 45° for 45 minutes. Sonicate again after heating is complete. Evaporate the solution using a stream of nitrogen, add 2.0 ml of internal standard solution and mix. *(NOTE — If incomplete dissolution is observed, discard the specimen).*

Internal standard solution. A 0.005 per cent w/v solution of *ethylparaben* in *dichloromethane*.

Reference solution. A 0.05 per cent w/v solution of *alprostadil IPRS* in *dehydrated alcohol*. Gently evaporate a 0.5 ml of the solution to dryness with a stream of nitrogen. Proceed as directed for the test solution beginning with "Add 150 µl of freshly prepared 4.0 per cent w/v solution....".

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with amino and cyano groups bonded to porous silica (5 µm),
- mobile phase: a mixture of 1000 volumes of *dichloromethane*, 6 volumes of *1,3-butanediol* and 0.5 volume of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The relative retention time with reference to alprostadil for ethylparaben is about 0.4

Inject the reference solution. The test is not valid unless the resolution between the peaks corresponding to alprostadil and the internal standard is not less than 9.0 and the relative standard deviation for replicate injections is not more than 2.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{34}O_5$ in the injection from the peak response ratios of alprostadil to the internal standard obtained with the reference solution and the test solution respectively.

Storage. Store protected from moisture, at a temperature of 2° to 8°, in single-dose containers, preferably of Type I glass.

Aluminium Acetate Ear Drops

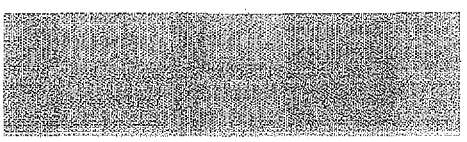
Aluminium Acetate Otic Drops; Aluminium Acetate Solution; Burow's Solution.

Aluminium Sulphate	255	g
Calcium Carbonate	100	g
Tartaric Acid	45	g
Glacial Acetic Acid	82.5	ml
Purified Water sufficient to produce	1000	ml

Dissolve the Aluminium Sulphate in 600 ml of Purified Water, add Glacial Acetic Acid followed by Calcium Carbonate mixed with the remainder of the Purified Water and allow to stand for not less than 24 hours in a cool place, stirring occasionally. Filter, add the Tartaric Acid to the filtrate and mix.

Aluminium Acetate Ear Drops contain not less than 1.7 per cent w/v and not more than 1.9 per cent w/v of aluminium, Al.

Description. A clear solution.



Tests

Weight per ml (2.4.29). 1.06 g to 1.08 g.

Other tests. Comply with the tests stated under Ear Drops.

Assay. Dilute 10.0 ml to 100.0 ml with *water*. To 10.0 ml of the resulting solution add 40.0 ml of *0.05 M disodium edetate*, 90 ml of *water* and 0.15 ml of *methyl red solution*. Neutralise by the addition of *1 M sodium hydroxide* dropwise and warm on a water-bath for 30 minutes. Cool, add 1 ml of *2 M nitric acid* and 5 g of *hexamine* and titrate with *0.05 M lead nitrate* using 0.5 ml of *xylenol orange solution* as indicator. Carry out a blank titration.

1 ml of *0.05 M disodium edetate* is equivalent to 0.001349 g of Al.

Storage. Store protected from light, in well-filled containers.

Dried Aluminium Hydroxide

Dried Aluminium Hydroxide Gel; Hydrated Aluminium Oxide

Dried Aluminium Hydroxide consists largely of hydrated aluminium oxide together with varying quantities of basic aluminium carbonate and bicarbonate.

Dried Aluminium Hydroxide contains not less than 47.0 per cent and not more than 60.0 per cent of Al_2O_3.

Description. A white, light, amorphous powder containing some aggregates.

Identification

A solution in *dilute hydrochloric acid* gives the reactions of aluminium salts (2.3.1).

Tests

pH (2.4.24). Not more than 10.0, determined in a 4.0 per cent w/v suspension in *carbon dioxide-free water*.

Arsenic (2.3.10). Dissolve 2 g in 18 ml of *brominated hydrochloric acid*, add 42 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (5 ppm).

Heavy metals (2.3.13). Dissolve 0.33 g in 10 ml of *dilute hydrochloric acid* with the aid of heat, filter if necessary, and dilute to 25 ml with *water*. The resulting solution complies with the limit test for heavy metals Method A (60 ppm).

Chlorides (2.3.12). Dissolve 0.1 g in 10 ml of *dilute nitric acid*, boil, cool, dilute to 100 ml with *water* and filter. 20 ml of the filtrate complies with the limit test for chlorides (1.25 per cent).

Sulphates (2.3.17). Dissolve 0.5 g in 5 ml of *dilute hydrochloric acid*, boil, cool, dilute to 200 ml with *water* and filter. 5 ml of the filtrate complies with the limit test for sulphates (1.2 per cent).

Neutralising capacity. Pass a sufficient quantity, triturated if necessary, through a sieve of nominal mesh aperture of 150 µm. Weigh 0.5 g of the sifted material and add to 200.0 ml of *0.05 M hydrochloric acid* previously heated to 37° and stir continuously, maintaining the temperature at 37°; the pH of the solution, at 37°, after 10, 15 and 20 minutes, is not less than 1.8, 2.3 and 3.0 respectively and at no time is more than 4.5. Add 10.0 ml of *0.5 M hydrochloric acid* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate with *0.1 M sodium hydroxide* to pH 3.5.

Not more than 35.0 ml of *0.1 M sodium hydroxide* is required and the pH of the solution at 37° at no time is more than 4.5.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 10^3 CFU and total fungal count is not more than 10^2 CFU per g determined by plate count. 1g is free from *bile-tolerant gram-negative bacteria* and *Escherichia coli*.

Assay. Dissolve 0.4 g in a mixture of 3 ml of *hydrochloric acid* and 3 ml of *water* by warming on a water-bath, cool to below 20° and dilute to 100.0 ml with *water*. To 20.0 ml of the solution, add 40.0 ml of *0.05 M disodium edetate*, 80 ml of *water*, and 0.15 ml of *methyl red solution* and neutralise by the dropwise addition of *1 M sodium hydroxide*. Warm on a water-bath for 30 minutes, add 3 g of *hexamine* and titrate with *0.05 M lead nitrate* using 0.5 ml of *xylenol orange solution* as indicator. Carry out a blank titration.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002549 g of Al_2O_3.

Storage. Store protected from moisture.

Aluminium Hydroxide Gel

Aluminium Hydroxide Suspension; Aluminium Hydroxide Mixture

Aluminium Hydroxide Gel is an aqueous suspension of hydrated aluminium oxide together with varying quantities of basic aluminium carbonate and bicarbonate. It may contain Glycerin, Sorbitol, Sucrose or Saccharin as sweetening agents and Peppermint Oil or other suitable flavours. It may also contain suitable antimicrobial agents.

Aluminium Hydroxide Gel contains not less than 3.5 per cent and not more than 4.4 per cent w/w of Al_2O_3.

Category. Antacid.

Description. A white, viscous suspension, translucent in thin layers; small amounts of clear liquid may separate on standing.

Identification

A solution in *dilute hydrochloric acid* gives the reactions of aluminium salts (2.3.1).

Tests

pH (2.4.24). 5.5 to 8.0.

Arsenic (2.3.10). Dissolve 10.0 g in 18 ml of *brominated hydrochloric acid*, add 42 ml of *water* and remove the excess bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). Dissolve 2.0 g in 10 ml of *dilute hydrochloric acid*, filter if necessary, and dilute to 25 ml with *water*. The resulting solution complies with the limit test for heavy metals, Method A (10 ppm).

Chlorides (2.3.12). Dissolve 0.5 g in 5 ml of *dilute nitric acid*, boil, cool, dilute to 100 ml with *water* and filter. 20 ml of the filtrate complies with the limit test for chlorides (0.25 per cent).

Sulphates (2.3.17). Dissolve 1.0 g in 5 ml of *dilute hydrochloric acid* with the aid of heat. Cool and dilute to 100 ml with *water*. Mix well and filter, if necessary. To 5 ml of the filtrate add 2 ml of *dilute hydrochloric acid*; the solution complies with the limit test for sulphates (0.3 per cent).

Neutralising capacity. Disperse 5.0 g in 100 ml of *water*, heat to 37°, add 100.0 ml of *0.1 M hydrochloric acid* previously heated to 37° and stir continuously, maintaining the temperature at 37°; the pH of the solution, at 37°, after 10, 15 and 20 minutes, is not less than 1.8, 2.3 and 3.0 respectively and at no time is more than 4.5. Add 10.0 ml of *0.5 M hydrochloric acid* previously heated to 37°, stir continuously for 1 hour maintaining the temperature at 37° and titrate with *0.1 M sodium hydroxide* to pH 3.5.

Not more than 50.0 ml of *0.1 M sodium hydroxide* is required.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 10^2 CFU per ml determined by plate count. 1 ml is free from *Escherichia coli*.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Dissolve 5.0 g in 3 ml of *hydrochloric acid* by warming on a water-bath; cool to below 20° and dilute to 100.0 ml with *water*. To 20.0 ml of the solution, add 40.0 ml of *0.05 M disodium edetate*, 80 ml of *water*, and 0.15 ml of *methyl red solution* and neutralise by the dropwise addition of *1 M sodium hydroxide*. Warm on a water-bath for 30 minutes, add 3 g of *hexamine* and titrate with *0.05 M lead nitrate* using 0.5 ml of *xylenol orange solution* as indicator. Carry out a blank titration.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002549 g of Al_2O_3.

Storage. Store at a temperature not exceeding 30°. Do not freeze.

Aluminium Magnesium Silicate

$Al_2MgO_8Si_2$ Mol. Wt. 262.4

Aluminium Magnesium Silicate is mixture of particles with colloidal particle size of montmorillonite and saponite, free from grit and non-swellable ore.

Aluminium Magnesium Silicate contains not less than 95.0 per cent and not more than 105.0 per cent each of the stated amount of aluminium, Al and magnesium, Mg, calculated on the dried basis.

Category. Pharmaceutical aid.

Description. A almost white powder, granules or plates.

Identification

A. Fuse 1 g with 2 g of *anhydrous sodium carbonate*. Warm the residue with *water* and filter. Acidify the filtrate with *hydrochloric acid* and evaporate to dryness on a water bath. About 0.25 g of the residue gives the reaction of silicates (2.3.1).

B. Dissolve the remainder of the residue obtained in identification test A in a mixture of 5 ml of *dilute hydrochloric acid* and 10 ml of *water*. Filter and add *ammonium chloride buffer solution pH 10*. A white, gelatinous precipitate is formed. Centrifuge and keep the supernatant for identification C. Dissolve the remaining precipitate in *dilute hydrochloric acid*; gives the reaction of aluminium (2.3.1).

C. The supernatant liquid obtained after centrifugation in identification test B gives the reaction of magnesium (2.3.1).

Tests

pH (2.4.24). 9.0 to 10.0, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water*.

Arsenic (2.3.10). Dissolve 16.6 g in 100 ml of *dilute hydrochloric acid* in a 250-ml beaker. Mix, cover with a watch glass and boil gently, with occasional stirring, for 15 minutes, allow the insoluble matter to settle and decant the supernatant liquid through a rapid-flow filter paper into a 250-ml volumetric flask, retaining as much sediment as possible in the beaker. To the residue in the beaker, add 25 ml of hot *dilute hydrochloric acid*, stir, heat to boiling, allow the insoluble matter to settle and decant the supernatant liquid through the filter into the volumetric flask. Repeat the extraction with 4 additional quantities, each of 25 ml, of hot *dilute hydrochloric acid*, decanting each supernatant liquid through the filter into the volumetric flask. At the last extraction, transfer as much of

the insoluble matter as possible onto the filter. Allow the combined filtrates to cool to room temperature and dilute to 250.0 ml with *dilute hydrochloric acid*. 50 ml of the resulting solution complies with the limit test for arsenic (3 ppm).

Lead. Not more than 15 ppm, determine by atomic absorption spectrophotometry (2.4.2), measuring at 217 nm using a oxidising air-acetylene flame.

Test solution. Dissolve 10 g in 100 ml of *dilute hydrochloric acid* in a 250-ml beaker. Mix, cover with a watch glass and boil for 15 minutes, cool to room temperature, allow the insoluble matter to settle. Decant the supernatant liquid through a rapid-flow filter paper into a 400-ml beaker. To the insoluble matter in the 250-ml beaker, add 25 ml of hot *water*. Stir, allow the insoluble matter to settle and decant the supernatant liquid through the filter into the 400-ml beaker. Repeat the extraction with 2 additional quantities, each of 25 ml of *water*, decanting each time the supernatant liquid through the filter into the 400-ml beaker. Wash the filter with 25 ml of hot *water*, collecting this filtrate in the 400-ml beaker. Concentrate the combined filtrates to about 20 ml by gently boiling. If a precipitate appears, add about 0.1 ml of *nitric acid*, heat to boiling and allow to cool to room temperature. Filter the concentrated extracts through a rapid-flow filter paper into a 50-ml volumetric flask. Transfer the remaining contents of the 400-ml beaker through the filter paper and into the flask with *water*. Dilute the solution to 50.0 ml with *water*.

Reference solution. Prepare the reference solution using *lead standard solution AAS (10 ppm Pb)*, diluted if necessary with *water*.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 10^3 CFU per g determined by plate count. 1 g is free from *Escherichia coli*.

Assay. *For aluminium*—Determine by atomic absorption spectrophotometry (2.4.2), measuring at 309 nm using a oxidising acetylene-nitrous oxide flame and aluminium hollow-cathode lamp.

Test solution. Mix 0.2 g with 1.0 g of *lithium metaborate* in a platinum crucible. Heat slowly at first and ignite at 1000 to 1200° for 15 minutes, cool, then place the crucible in a 100-ml beaker containing 25 ml of *dilute nitric acid* and add an additional 50 ml of *dilute nitric acid*, filling and submerging the crucible. Place a polytetrafluoroethylene-coated magnetic stirring bar in the crucible and stir gently with a magnetic stirrer until dissolution is complete. Pour the contents into a 250-ml beaker and remove the crucible. Warm the solution and transfer through a rapid-flow filter paper into a 250-ml volumetric flask, wash the filter and beaker with *water* and dilute to 250.0 ml with *water* (solution A). To 20.0 ml of solution A, add 20 ml of a 1.0 per cent w/v solution of *sodium chloride* and dilute to 100.0 ml with *water*.

Reference solution. Dissolve, with gentle heating, 1.0 g of *aluminium* in a mixture of 10 ml of *hydrochloric acid* and 10 ml of *water*, cool. Dilute to 1000.0 ml with *water* (1 mg of aluminium per millilitre). Into 3 identical volumetric flasks, each containing 0.2 g of *sodium chloride*, introduce 2.0 ml, 5.0 ml and 10.0 ml of the solution respectively, and dilute to 100.0 ml with *water*.

For magnesium—Determine by atomic absorption spectrophotometry (2.4.2), measuring at 285 nm using a reducing air acetylene flame and magnesium hollow-cathode lamp.

Test solution. Dilute 25.0 ml of solution A, prepared in the assay for aluminium, to 50.0 ml with *water*. To 5.0 ml of the solution add 20.0 ml of *lanthanum nitrate solution* and dilute to 100.0 ml with *water*.

Reference solution. Place 1.0 g of *magnesium* in a 250-ml beaker containing 20 ml of *water* and carefully add 20 ml of *hydrochloric acid*, warming if necessary to dissolve. Transfer the solution to a volumetric flask and dilute to 1000.0 ml with *water* (1 mg of magnesium per millilitre). Dilute 5.0 ml of the solution to 250.0 ml with *water*. Into 4 identical volumetric flasks, introduce 5.0 ml, 10.0 ml, 15.0 ml and 20.0 ml of the solution respectively. To each flask add 20.0 ml of *lanthanum nitrate solution* and dilute to 100.0 ml with *water*.

Labelling. The label states the content of aluminium and magnesium.

Aluminium, Magnesium and Simethicone Oral Suspension

Aluminium Hydroxide, Magnesium Hydroxide and Simethicone Oral Suspension

Aluminium, Magnesium and Simethicone Oral Suspension contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aluminium hydroxide, $Al(OH)_3$ and magnesium hydroxide, $Mg(OH)_2$ and polydimethylsiloxane $[-(CH3)_2SiO-]_n$ is not less than 85.0 per cent and not more than 115.0 per cent of the stated amount of simethicone.

Usual strengths. Aluminium Hydroxide, 250 mg, Magnesium Hydroxide, 250 mg and Simethicone, 50 mg per 5 ml; Aluminium Hydroxide, 200 mg, Magnesium Hydroxide, 200 mg and Simethicone, 25 mg per 5 ml.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with

polydimethylsiloxane IPRS or with the reference spectrum of polydimethylsiloxane.

B. Dissolve about 5.0 g of oral suspension in 10.0 ml of *3 M hydrochloric acid*, add 5 drops of *methyl red solution* and heat to boiling. Add *6M ammonium hydroxide solution* until the colour changes to deep yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

C. Wash the precipitate obtained in test B with 0.002 per cent w/v solution of hot *ammonium chloride solution* and dissolve the precipitate in *hydrochloric acid*. Divide the resulting solution into two equal portions. The dropwise addition of *6 M ammonium hydroxide solution* to first portion yields a gelatinous white precipitate, which does not dissolve in an excess of *6 M ammonium hydroxide solution*. The dropwise addition of *1 M sodium hydroxide* to the second portion yields a gelatinous white precipitate, which dissolves in an excess of *1 M sodium hydroxide*, leaving some turbidity.

Tests

pH (2.4.24). 7.0 to 8.6.

Neutralising capacity. Transfer an accurately weighed quantity of the uniform mixture, equivalent to the minimum labelled dosage, to a 250-ml beaker, add *water* to make a total volume of about 70 ml, and mix on the magnetic stirrer for 1 minute.

Further add 30.0 ml of *1.0 M hydrochloric acid* while continuing to stir with the magnetic stirrer for 10 minutes, after the addition of the acid, then begin to titrate immediately, titrate the excess of *hydrochloric acid* with *0.5 M sodium hydroxide* to obtain a pH of 3.5. Calculate the number of mEq of acid consumed by the solution using the formula:

$$\text{Total mEq} = (30 \times M_{HCl}) - (V_{NaOH} \times M_{NaOH})$$

in which M_{HCl} and M_{NaOH} are the molarities of the *hydrochloric acid* and the *sodium hydroxide* respectively; and V_{NaOH} is the volume of sodium hydroxide used for titration. Express the result in mEq of acid consumed per g of the substance tested.

Acceptance criteria. The acid consumed by the minimum single dose recommended in the labelling is not less than 5 mEq and not less than the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (FA \times A) + 0.8 \times (FM \times M)$$

in which FA and FM are theoretical acid-neutralizing capacity of aluminum hydroxide [$Al(OH)_3$], 0.0385 mEq and theoretical acid-neutralizing capacity of magnesium hydroxide [$Mg(OH)_2$], 0.0343 mEq respectively, A and M are the amount of aluminum hydroxide [$Al(OH)_3$] in the sample, based on the stated quantity (mg) and amount of magnesium hydroxide [$Mg(OH)_2$] in the sample, based on the stated quantity (mg) respectively.

Other tests. Comply with the tests stated under Oral Liquids.

Microbial contamination (2.2.9). The total aerobic viable count is not more than 10^2 CFU per g. It meets the requirements of the tests for the absence of *Escherichia coli*.

Assay

Aluminium hydroxide — Transfer a measured volume containing about 0.8 g of the Aluminium Hydroxide to a suitable beaker. Add 20 ml of *water*, stir, and slowly add 10 ml of *hydrochloric acid*. Heat gently, if necessary, cool to room temperature, and filter into a 200.0 ml volumetric flask. Wash the filter with *water* into the flask, and add *water* to volume (solution A).

Pipet 10 ml of the solution A into a 250-ml beaker, add 20 ml of *water*, then add with continuous stirring, 25.0 ml of *0.05 M disodium edetate* and 20 ml of *acetic acid–ammonium acetate buffer*, and heat the solution near the boiling temperature for 5 minutes. Cool, add 50 ml of *alcohol* and 2 ml of *dithizone*. Titrate the excess *disodium edetate* with *0.05 M zinc sulphate* until the colour changes from green-violet to rose-pink. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* is consumed.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

1 mg of dried aluminium hydroxide gel is equivalent to 0.765 mg of $Al(OH)_3$.

Magnesium hydroxide — Pipet a volume of the solution A containing 40 mg of Magnesium Hydroxide into a 400-ml beaker, add 200.0 ml of *water* and 20 ml of *triethanolamine*, and stir. Add 10 ml of *ammonia–ammonium chloride buffer* and 3 drops of an *eriochrome black indicator solution* prepared by dissolving 200 mg of *eriochrome black* in a mixture of 15 ml of *triethanolamine* and 5 ml of *alcohol*, and mixing. Cool the solution between 3° to 4° by immersion of the beaker in an ice bath, then remove, and titrate with *0.05 M disodium edetate* to a blue endpoint. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* is consumed.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

Polydimethylsiloxane — Determine by infrared absorption spectrophotometry (2.4.6).

Blank. Mix 10 ml of *toluene* with 0.5 g of *anhydrous sodium sulphate*, and centrifuge to obtain a clear supernatant.

Test solution. Transfer a measured volume containing about 50 mg of Simethicone to a suitable round, narrow-mouth, screw-capped, 100 ml bottle. Add 40 ml of *0.1 M sodium hydroxide*, and swirl to disperse. Add 25.0 ml of *toluene*, close the bottle securely with a cap having an inert liner, and shake

for 30 minutes on a reciprocating shaker. Transfer the mixture to a 125-ml separator, and allow to separate. Remove the upper, organic layer to a screw-capped, centrifuge tube containing 0.5 g of *anhydrous sodium sulphate*. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

Reference solution. Weigh accurately about 50.0 mg of *polydimethylsiloxane IPRS* to a suitable round, narrow-mouth add 40 ml of *0.1 M sodium hydroxide*, and swirl to disperse. Add 25.0 ml of *toluene*, close the bottle securely with a cap having an inert liner, and shake for 30 minutes on a reciprocating shaker. Transfer the mixture to a 125-ml separator, and allow to separate. Remove the upper, organic layer to a screw-capped, centrifuge tube containing 0.5 g of *anhydrous sodium sulphate*. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

Measure the absorbance by using 0.5-mm cell at the wavelength of maximum absorbance at about 7.9 µm, with an infrared absorption spectrophotometer.

Calculate the content of $[-(CH3)_2SiO-]_n$ in the oral suspension.

Sodium content — Determine by atomic absorption spectrophotometery (2.4.2), equipped with a sodium hollow-cathode lamp and an air–acetylene flame.

Blank. A mixture of 4.0 ml of *1 M hydrochloric acid* and 10.0 ml of *potassium chloride solution* in a 100-ml volumetric flask, and dilute with *water* to volume.

Potassium chloride solution. A solution containing *3.8 per cent w/v* of *potassium chloride* in *water*.

Sodium chloride solution. Weigh 254.2 mg of *sodium chloride* (previously dried at 105° for 2 hours) to a 100-ml volumetric flask and dilute to 100.0 ml with *water*. Dilute 1.0 ml of the solution to 100.0 ml with *water*. This solution contains 10 µg of sodium (equivalent to 25.42 µg of sodium chloride) per ml.

Test solution. Transfer 5.0 ml of Oral Suspension to a 100-ml volumetric flask. Add 50 ml of *1 M hydrochloric acid*, boil for 15 minutes, cool to room temperature, and dilute with *water* to volume. Filter, discarding the first few ml of the filtrate. Transfer 5.0 ml of the filtrate to a 100-ml volumetric flask containing 10.0 ml of *Potassium chloride solution*, and dilute with *water* to volume.

Reference solution. Transfer 4.0 ml of *1 M hydrochloric acid* and 10.0 ml of *potassium chloride solution* in a two 100-ml volumetric flask.. To the respective flask, add 5.0 ml and 10.0 ml of *sodium chloride solution* and, dilute to volume with *water*. The reference solutions contain 0.5 µg and 1.0 µg sodium per ml respectively.

Determine the absorbance of the reference solutions and the test solution at the sodium emission line of 589.0 nm. Plot the absorbance of the reference solution versus concentration, in µg per ml, of sodium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration in µg per ml of sodium in the test solution.

Calculate the quantity, in mg, of Na in oral suspension taken by the formula:

$$(1/N) \times C \times D \times F$$

Where, N is the volume of the oral suspension taken to prepare the test solution, C is the concentration, in µg per ml, of sodium in the test solution, D is the dilution factor for the test solution, 2000 and F is the conversion factor, 0.001 mg per µg.

Storage. Store in tightly-closed containers and avoid freezing.

Labelling. The label states (1) the oral suspension to be labelled to state the sodium content, if it is more than 1 mg per ml; (2) The oral suspension may be labelled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel.

Aluminium, Magnesium and Simethicone Chewable Tablets

Aluminium Hydroxide, Magnesium Hydroxide and Simethicone Chewable Tablets

Aluminium, Magnesium and Simethicone Chewable Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aluminium hydroxide $[Al(OH)_3]$ and magnesium hydroxide$[Mg(OH)_2]$ and polydimethylsiloxane$[-(CH3)_2SiO-]_n$ is not less than 85.0 per cent and not more than 115.0 per cent of the stated amount of simethicone.

Usual strengths. Aluminium Hydroxide, 300 mg, Magnesium Hydroxide, 200 mg and Simethicone, 25 mg; Aluminium Hydroxide, 250 mg, Magnesium Hydroxide, 250 mg and Simethicone, 50 mg; Aluminium Hydroxide, 300 mg, Magnesium Hydroxide, 150 mg and Simethicone, 40 mg; Aluminium Hydroxide, 300 mg, Magnesium Hydroxide, 300 mg and Simethicone, 25 mg.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *polydimethylsiloxane IPRS* or with the reference spectrum of polydimethylsiloxane.

B. Dissolve about 0.6 g of magnesium hydroxide in 25.0 ml of *3 M hydrochloric acid*, add 25 ml of *water* and mix. Boil gently for 2 minutes. Allow to cool, and filter, add 5 drops of *methyl red solution* and heat to boiling. Add *6M ammonia hydroxide*

solution until the colour changes to deep yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

C. Wash the precipitate obtained in test B with 0.002 per cent w/v solution of hot *ammonium chloride* and dissolve the precipitate in *hydrochloric acid*. Divide the resulting solution into two equal portions. The dropwise addition of *6 M ammonium hydroxide solution* to first portion yields a gelatinous white precipitate, which does not dissolve in an excess of *6 M ammonium hydroxide solution*. The dropwise addition of *1 M sodium hydroxide* to the second portion yields a gelatinous white precipitate, which dissolves in an excess of *1 M sodium hydroxide*, leaving some turbidity.

Tests

Neutralising capacity. Weigh and powder 20 tablets, disperse one tablet in 250-ml of beaker, add 50.0 ml of *water* and mix on the magnetic stirrer for 1 minute. If wetting is desired, add not more than 5 ml of *alcohol* (neutralized to a pH of 3.5), and mix to wet the substance thoroughly. Add 70 ml of *water*, and mix on the magnetic stirrer for 1 minute. Further add 30.0 ml of *1 M hydrochloric acid* while continuing to stir with the magnetic stirrer for 10 minutes, after the addition of the acid. Discontinue stirring briefly, and without delay remove any gum base from the beaker using a long needle. Promptly rinse the needle with 20 ml of *water*, collecting the washing in the beaker, and further stirring for 5 minutes, then begin to titrate immediately, titrate the excess *hydrochloric acid* with *0.5 M sodium hydroxide* to obtain a pH of 3.5. Calculate the number of mEq of acid is consumed by the Tablet using the formula:

$$\text{Total mEq} = (30 \times M_{HCl}) - (V_{NaOH} \times M_{NaOH})$$

in which M_{HCl} and M_{NaOH} are the molarities of the *hydrochloric acid* and the *sodium hydroxide* respectively; and V_{NaOH} is the volume of sodium hydroxide used for titration. Express the result in mEq of acid consumed per g of the substance tested.

Acceptance criteria. The acid consumed by the minimum single dose recommended in the labelling is not less than 5 mEq and not less than the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (FA \times A) + 0.8 \times (FM \times M)$$

in which FA and FM are theoretical acid-neutralizing capacity of aluminum hydroxide [$Al(OH)_3$], 0.0385 mEq and theoretical acid-neutralizing capacity of magnesium hydroxide [$Mg(OH)_2$], 0.0343 mEq respectively, A and M are the amount of aluminum hydroxide [$Al(OH)_3$] in the sample, based on the stated quantity (mg) and amount of magnesium hydroxide [$Mg(OH)_2$] in the sample, based on the stated quantity (mg) respectively.

Other tests. Comply with the tests stated under Tablets.

Assay

Aluminium hydroxide — Weigh and powder 20 tablets. Transfer a portion of the powder containing about 0.8 g of *aluminum hydroxide*, to a 150-ml beaker. Add 20 ml of *water*, stir, and slowly add 30 ml of *3 M hydrochloric acid*. Heat gently, if necessary, cool to room temperature, and filter into a 200-ml volumetric flask. Wash the filter with *water* into the flask, and add *water* to volume (solution A).

Pipet 10 ml of the solution A into a 250-ml beaker, add 20 ml of *water*, then add with continuous stirring, 25.0 ml of *0.05 M disodium edetate* and 20 ml of *acetic acid–ammonium acetate buffer*, and heat the solution near the boiling temperature for 5 minutes. Cool, add 50 ml of *alcohol* and 2 ml of *dithizone*. Titrate the excess *disodium edetate* with *0.05 M zinc sulphate* until the colour changes from green-violet to rose-pink. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* is consumed.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

1 mg of dried aluminium hydroxide gel is equivalent to 0.765 mg of $Al(OH)_3$.

Magnesium hydroxide — Pipet a volume of the solution A containing 40 mg of *magnesium hydroxide*, into a 400-ml beaker, add 200.0 ml of *water* and 20 ml of *triethanolamine*, and stir. Add 10 ml of *ammonia–ammonium chloride buffer* and 3 drops of an *eriochrome black indicator solution* prepared by dissolving 200 mg of *eriochrome black* in a mixture of 15 ml of *triethanolamine* and 5 ml of *alcohol*, and mixing). Cool the solution between 3° to 4° by immersion of the beaker in an ice bath, then remove, and titrate with *0.05 M disodium edetate* to a blue endpoint. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* is consumed.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

Polydimethylsiloxane — Determine by infrared absorption spectrophotometry (2.4.6).

Blank. Mix 10 ml of *toluene* with 1 g of *anhydrous sodium sulphate*, and centrifuge to obtain a clear supernatant.

Test solution. Weigh and powder 20 Tablets. Transfer a portion of the powder containing 33 mg of Simethicone, to a suitable round, narrow-mouth, screw-capped, 120-ml bottle. Add 40 ml of *0.1 M sodium hydroxide*, and swirl to disperse. Add 20.0 ml of *toluene*, close the bottle securely with a cap having an inert liner, and shake for 30 minutes on a reciprocating shaker. Transfer the mixture to a 125-ml separator, and allow to separate. Remove the upper, organic layer to a screw-capped, centrifuge tube containing 2 g of *anhydrous sodium sulphate*.

Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

Reference solution. Weigh about 33 mg of *Polydimethylsiloxane IPRS* to a suitable round, narrow-mouth, screw-capped, 120-ml bottle. Add 40 ml of *0.1 M sodium hydroxide*, and swirl to disperse. Add 20.0 ml of *toluene*, close the bottle securely with a cap having an inert liner, and shake for 30 minutes on a reciprocating shaker. Transfer the mixture to a 125-ml separator, and allow to separate. Remove the upper, organic layer to a screw-capped, centrifuge tube containing 2 g of *anhydrous sodium sulphate*. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

Measure the absorbance by using 0.5-mm cell at the wavelength of maximum absorbance at about 1265.8 cm^{-1} with an infrared absorption spectrophotometer.

Calculate the content of $[-(CH3)_2SiO-]_n$ in the tablets.

Sodium content — Determine by atomic absorption spectrophotometery (2.4.2), equipped with a sodium hollow-cathode lamp and an air–acetylene flame.

Blank. A mixture of 4.0 ml of *1 M hydrochloric acid* and 10.0 ml of *potassium chloride solution* in a 100.0 ml volumetric flask, and dilute with *water* to volume.

Potassium chloride solution. A solution containing *3.8 per cent w/v of potassium chloride* in *water.*

Sodium chloride solution. Dissolve 254.2 mg of *sodium chloride* (previously dried at 105° for 2 hours) to a 100.0 ml volumetric flask and dilute to 100.0 ml with *water*. Dilute 1.0 ml of the solution to 100.0 ml with *water*. This solution contains 10 µg of sodium (equivalent to 25.42 µg of sodium chloride) per ml.

Test solution. Weigh and powder 20 Tablets. Transfer a portion of the powder, equivalent to the average weight of one Tablet, to a 100-ml volumetric flask. Add 50 ml of *1 M hydrochloric acid*, boil for 15 minutes, cool to room temperature, and dilute with *water* to volume. Filter, discarding the first few ml of the filtrate. Transfer 5.0 ml of the filtrate to a 100-ml volumetric flask containing 10.0 ml of *potassium chloride solution*, and dilute with *water* to volume.

Reference solution. Transfer 4.0 ml of *1 M hydrochloric acid* and 10.0 ml of *potassium chloride solution* in a two 100.0 ml volumetric flask.. To the respective flask, add 5.0 ml and 10.0 ml of *sodium chloride solution* and, dilute to volume with *water*. The reference solutions contain 0.5 µg and 1.0 µg sodium per ml respectively.

Determine the absorbances of the reference solutions and the test solution at the sodium emission line of 589.0 nm. Plot the absorbance of the reference solution versus concentration, in µg per ml, of sodium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration in µg per ml of sodium in the test solution.

Calculate the quantity, in mg, of Na in the tablets taken by the formula:

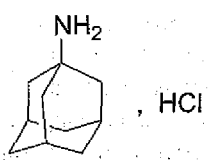
$$C \times D \times F$$

where C is the concentration, in µg per ml, of sodium in the test solution, D is the dilution factor for the Sample solution, 2000 and F is the conversion factor, 0.001 mg per µg.

Storage. Store in tightly-closed containers.

Labelling. The label states (1) the Chewable Tablets to indicate that they are to be chewed before being swallowed; (2) the Chewable Tablets to be labelled to state the sodium content if it is more than 5 mg per Tablet; (3) The Chewable Tablets may be labelled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminium hydroxide gel.

Amantadine Hydrochloride



$C_{10}H_{17}N,HCl$ Mol. Wt. 187.7

Amantadine Hydrochloride is tricyclo[3.3.1.$1^{3,7}$]dec-1-ylamine hydrochloride.

Amantadine Hydrochloride contains not less then 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{17}N,HCl$, calculated on the anhydrous basis.

Category. Antiviral; antiparkinsonian.

Description. A white or almost white, crystalline powder; sublimes when heated.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Dissolve 0.1 g in 5 ml of *water*, add 0.5 ml of *5 M sodium hydroxide*, extract with 5 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate* with 2 ml of *dichloromethane* and evaporate the solution to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amantadine hydrochloride IPRS* treated in the same manner or with the reference spectrum of amantadine.

B. Dissolve 0.2 g in 1 ml of *0.1 M hydrochloric acid* and add 1 ml of a 50 per cent w/v solution of *sodium nitrite*; a white precipitate is produced.

C. 1 ml of a 10 per cent w/v solution in *carbon dioxide-free water* gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 3.0 to 5.5, determined in a 20.0 per cent w/v solution.

Related substances. Determine by gas chromatography (2.4.13).

Internal standard solution. Dissolve 0.5 g of adamantane in *dichloromethane* and dilute to 10.0 ml with the same solvent.

Test solution. Weigh 0.5 g of the substance under examination into a centrifuge tube. Add 9 ml of *dichloromethane* and 10 ml of a 21.0 per cent w/v solution of *sodium hydroxide*. Shake for 10 minutes. Discard the upper layer. Dry the lower layer over *anhydrous sodium sulphate*. Filter and collect the filtrate in a volumetric flask. Add 0.1 ml of the internal standard solution and dilute to 10.0 ml with *dichloromethane*.

Reference solution. Weigh 5 mg of *amantadine hydrochloride IPRS* into a centrifuge tube. Add 9 ml of *dichloromethane* and 10 ml of a 21.0 per cent w/v solution of *sodium hydroxide*. Shake for 10 minutes. Discard the upper layer. Dry the lower layer over *anhydrous sodium sulphate*. Filter and collect the filtrate in a volumetric flask. Add 1.0 ml of the internal standard solution and dilute to 100.0 ml with *dichloromethane*.

Chromatographic system

- a capillary column 30 m x 0.53 mm packed with base-deactivated poly(dimethyl)(diphenyl)siloxane (film thickness 1 µm),
- temperature:

column	time (in min.)	temperature (°)
	0 – 5	70
	5 – 23	70 – 250
	23 – 40	250

  inlet port: 220° and detector: 300°,
- flow rate: 4 ml per minute, using helium as the carrier gas,
- Split ratio: 1:50.

The relative retention time with reference to amantadine (retention time: about 14 minutes) for adamantane is about 0.8.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to amantadine and adamantane is not less than 5.0.

Inject 1 µl of the reference solution and the test solution.

Any secondary peak. Calculate the ratio (R_1) of the area of the peak due to amantadine to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with the test solution, calculate the ratio of the area of any secondary peak to the area of the peak due to the internal standard: this ratio is not more than R_1 (0.10 per cent).

The sum of all the secondary peaks. Calculate the ratio (R_2) of 3 times the area of the peak due to amantadine to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with the test solution, calculate the ratio of the sum of the areas of any peaks, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: this ratio is not more than R_2 (0.3 per cent);

disregard limit: Calculate the ratio (R_3) of 0.5 times the area of the peak due to amantadine to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution; from the chromatogram obtained with the test solution, calculate the ratio of the area of any peak, apart from the principal peak and the peak due to the internal standard, to the area of the peak due to the internal standard: disregard any peak with a ratio less than R_3 (0.05 per cent).

Heavy metals (2.3.13). A solution prepared by dissolving 1.0 g in 1 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 2.0 g.

Assay. Dissolve 0.15 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (2.4.25). Record the volume used between the two inflections.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01877 g of $C_{10}H_{17}N,HCl$.

Amantadine Capsules

Amantadine Hydrochloride Capsules

Amantadine Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amantadine hydrochloride, $C_{10}H_{17}N,HCl$.

Usual strength. 100 mg.

Identification

Dissolve the contents of capsules containing 0.1 g of Amantadine Hydrochloride in 5 ml of *water*, add 0.5 ml of *5 M sodium hydroxide*, extract with 5 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate* with 2 ml of *dichloromethane* and evaporate the solution to dryness. The residue complies with the following test.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amantadine hydrochloride IPRS* treated in the same manner or with the reference spectrum of amantadine.

B. Dissolve 0.2 g in 1 ml of *0.1 M hydrochloric acid* and add 1 ml of a 50 per cent w/v solution of *sodium nitrite*; a white precipitate is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *water;*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.0054 per cent w/v solution of *naphthalene* in *hexane*.

Test solution. Transfer 15.0 ml of the filtrate in 50-ml screw-capped test tube. Add 5.0 ml of *5M sodium hydroxide* and 10.0 ml of the internal standard solution into the test tube, and shake for 60 minutes. Collect the hexane layer.

Reference solution (a). A 0.01per cent w/v solution of *amantadine hydrochloride IPRS* in *water*.

Reference solution (b). Transfer 15.0 ml of reference solution (a) in 50-ml screw-capped test tube. Add 5.0 ml of *5M sodium hydroxide* and 10.0 ml of the internal standard solution into the test tube, and shake for 60 minutes. Collect the hexane layer.

Chromatographic system
- a capillary column 30 m x 0.32 mm, coated with a 0.25-µm film of dimethyl polysiloxane oil,
- temperature:
  column.100° for 3 minutes, 100° to 200° at a constant rate of 10° per minute, hold for 2 minutes,
- inlet port 250°and detector 300°,
- flow rate: 1.4 ml per minute using nitrogen as carrier gas,
- injection volume: 2 µl.

The relative retention time with respect to amantadine for naphthalene is about 0.9.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to naphthalene and amantadine is not less than 2.0, the tailing factor is not more than 2.0 for the amantadine peak and the relative standard deviation for replicate injections is not more than 2.0 per cent for the peak response ratio of amantadine to naphthalene.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{10}H_{17}N$, HCl in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{10}H_{17}N$, HCl.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Dissolve a quantity of the contents of capsules containing 0.1 g of Amantadine Hydrochloride in 2 ml of *water*, add 2 ml of a 20 per cent w/v solution of *sodium hydroxide* and 2 ml of *chloroform* and shake for 10 minutes. Separate the chloroform layer, dry over *anhydrous sodium sulphate* and filter.

Chromatographic system
- a glass column 1.8 m x 2 mm, packed with material prepared in the following manner. Mix 19.5 g of silanised diatomaceous support (such as Chromosorb G/AW/DMCS) with 60 ml of a 0.33 per cent w/v solution of *potassium hydroxide* in *methanol* and evaporate the solvent under reduced pressure while slowly rotating the mixture. Dissolve over a 5-hour period 0.4 g of low-vapour pressure hydrocarbons (type L) (such as Apiezon L) in 60 ml of *toluene*, add the solution to the prepared silanised diatomaceous support and evaporate the solvent under reduced pressure while slowly rotating the mixture,
- temperature:
  column. 100° to 200° at a constant rate of 6° per minute, inlet port. 220° and detector. 300°,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl or other suitable volume of the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. The area of any secondary peak is not more than 0.3 per cent and the sum of the areas of all secondary peaks is not more than 1.0 per cent, calculated by area normalization method.

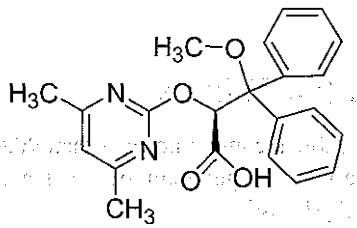
Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing 0.12 g of Amantadine Hydrochloride and warm in a mixture of 30 ml of *anhydrous glacial acetic acid* and 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01877 g of $C_{10}H_{17}N$,HCl.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Ambrisentan



$C_{22}H_{22}N_2O_4$ Mol. Wt. 378.4

Ambrisentan (2*S*)-2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3-methoxy-3,3-diphenylpropanoic acid.

Ambrisentan contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{22}N_2O_4$, calculated on the anhydrous basis.

Category. Antihypertensive.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ambrisentan IPRS* or with the reference spectrum of ambrisentan.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of equal volumes of mobile phase A and mobile phase B.

Test solution. Dissolve 25 mg of the substance under examination in 12.5 ml of mobile phase B and dilute to 25.0 ml with mobile phase A.

Reference solution (a). Dissolve 15 mg of the *diphenylvinyloxy impurity IPRS* in 5 ml of mobile phase B and dilute to 10.0 ml with mobile phase A.

Reference solution (b). Dissolve 25 mg of *ambrisentan IPRS* in 12.5 ml of mobile phase B and dilute to 25.0 ml with mobile phase A.

Reference solution (c). Mix 5.0 ml each of reference solution (a) and reference solution (b) and dilute to 50.0 ml with solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with solvent mixture. Further dilute 5.0 ml of the solution to 50.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm), (Such as Inertsil ODS 3),
- column temperature: 40°,
- mobile phase: A. buffer solution prepared by dissolving 1.38g of *sodium dihydrogen phosphate dihydrate* in 1000 ml of *water*, adjusted to pH 3.0 with *ortho-phosphoric acid*,

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
3	65	35
10	50	50
25	30	70
30	30	70
30.1	65	35
35	65	35

Inject reference solution (c). The test is not valid unless the tailing factor for the principal peak and diphenylvinyloxy impurity is not more than 2.0, the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 5.0 per cent each. Retention time of the principal peak is about 16 minutes and the relative retention time of diphenyl vinyloxy impurity is about 1.92

Inject reference solution (c) and the test solution in the chromatogram obtained with test solution, the area of any peak due to diphenylvinyloxy impurity is not more than the area of corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent). In the chromatogram obtained with test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The sum of areas of all the secondary peaks is not more than ten times the area of the principal peak in the chromatogram with reference solution (c) (1.0 per cent).

Enantiomeric purity. Not more than 2.0 per cent of *R*-isomer.

NOTE — prepare the solutions immediately before use.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 50.0 ml of *ethanol* and dilute to 100.0 ml with the *n-hexane*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, such as chiral pak AD-H (5 µm),
- mobile phase: a mixture of 90 volumes of *n-hexane*, 10 volumes of *ethanol*, 0.1 volume of *trifluoroacetic acid*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject the test solution. The test is not valid unless the column efficiency is not less than 1200 theoretical plates. The relative retention time with reference to ambrisentan (retention time: about 6 minutes) for *R*-isomer is about 1.3.

Calculate the content of *R*-isomer in ambrisentan by area normalization.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.4.43). Not more than 0.5 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of equal volumes of buffer solution and *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in 12.5 ml of *acetonitrile* and dilute to 25.0 ml with buffer solution. Dilute 10.0 ml of the solution to 100.0 ml with solvent mixture.

Reference solution. Dissolve 25 mg of *ambrisentan IPRS* in 12.5 ml of *acetonitrile* and dilute to 25.0 ml with buffer solution. Dilute 10.0 ml of the solution to 100.0 ml with solvent mixture.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm), (Such as Hypersil BDS C18),
- column temperature: 40°,
- mobile phase. a mixture of 55 volumes of a buffer solution prepared by dissolving 1.38 g of *sodium dihydrogen phosphate dihydrate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 45 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 5 µl.

The retention time of the principal peak is about 5 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{22}N_2O_4$.

Storage. Store protected from light and moisture.

Ambrisentan Tablets

Ambrisentan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ambrisentan, $C_{22}H_{22}N_2O_4$.

Usual Strengths. 5 mg; 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium : 900 ml of 0.05 M acetate buffer pH 5.0, prepared by dissolving 4.1 g of *sodium acetate* in sufficient *water* to produce 1000 ml, adjusted to pH 5.0 with *glacial acetic acid*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate.

Determine by liquid chromatography (2.4.14).

Buffer solution A. 0.01 M sodium dihydrogen phosphate dihydrate, adjusted to pH 3.0 with 10 per cent *orthophosphoric acid solution* and filter.

Solvent mixture. 25 volumes of buffer solution and 75 volumes of *acetonitrile*.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *ambrisentan IPRS* in solvent mixture and dilute quantitatively with the dissolution medium to obtain a solution of similar concentration as the test solution.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3 µm) (Such as Hypersil BDS C18),
- column temperature: 40°,
- mobile phase: A. a mixture of 85 volumes of buffer solution and 15 volumes of *acetonitrile*,

B. a mixture of 15 volumes of buffer solution and 85 volumes of *acetonitrile*,

- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 262 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	40	60
3	0	100
3.1	40	60
6.0	40	60

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{22}N_2O_4$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{22}H_{22}N_2O_4$.

Other tests. Complies with the tests stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of ambrisentan in 5 ml of *water* and then in sufficient volume of solvent mixture, mix with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.005 per cent w/v solution of *ambrisentan IPRS* in the solvent mixture.

Reference solution (b). A 0.0025 per cent w/v solution of *diphenylvinyloxy IPRS* in the solvent mixture.

Reference solution (c). Dilute 5.0 ml, each of, reference solution (a) and reference solution (b) to 100.0 ml with the solvent mixture.

Reference solution (d). A solution containing 0.025 per cent w/v of *ambrisentan IPRS* in solvent mixture, add 5 ml of reference solution (b).

Use the chromatographic system as described under Dissolution with the following modification.

- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
3	65	35
12	50	50
30	40	60
30.1	65	35
35	65	35

Inject reference solution (c) and (d). The test is not valid unless the resolution between ambrisentan and diphenylvinyloxy is not less than 25 in the chromatogram obtained with reference solution (d) and the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0 in the chromatogram obtained with reference solution (c).

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to diphenylvinyloxy is not more than 0.5 times than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of any other secondary peak is not more than 0. 5 times the area of the principal peak in the chromatogram obtained with the reference solution (c) (0. 5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

Test solution. Disperse 1 tablet in 2 ml of *water* with the aid of ultrasound for about 2 minutes. Add about 15 ml of solvent mixture and ultrasound for further 10 minutes, cool and dilute to 20.0 ml with the solvent mixture. Shake well to mix and filter, rejecting the first few ml of filtrate. Dilute further with the solvent mixture, if necessary.

Reference solution. A 0.025 per cent w/v solution of *ambrisentan IPRS* in the solvent mixture.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{22}N_2O_4$ in the tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of Ambrisentan in 5 ml of *water* add a sufficient volume of solvent mixture, with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with the solvent mixture.

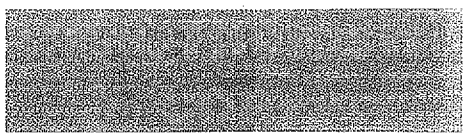
Reference solution. A 0.025 per cent w/v solution of *ambrisentan IPRS* in the solvent mixture.

Chromatographic system

Use the chromatographic system as described under Dissolution with the following modification.

- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

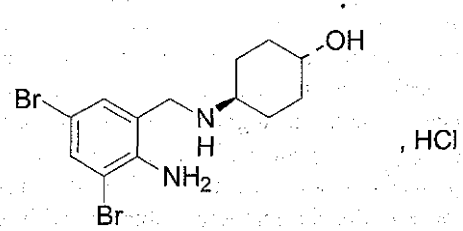


Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{22}N_2O_4$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Ambroxol Hydrochloride



$C_{13}H_{18}Br_2N_2O,HCl$ Mol. Wt. 414.6

Ambroxol hydrochloride is *trans*-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol hydrochloride.

Ambroxol Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{13}H_{18}Br_2N_2O,HCl$, calculated on the dried basis.

Category. Mucolytic.

Description. A white or yellowish crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ambroxol hydrochloride IPRS* or with the reference spectrum of ambroxol hydrochloride.

B. Dissolve 25 mg in 2.5 ml of *water*, add 1.0 ml of *dilute ammonia* and allow to stand for 5 minutes. Acidify the aqueous layer with *dilute nitric acid* and filter. The filtrate gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *water* and dilute to 50.0 ml with the same solvent.

Reference solution (a). Dissolve 5 mg of *ambroxol hydrochloride IPRS* in 250.0 ml of *water*. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of the substance under examination in 0.2 ml of *methanol* and add 0.04 ml of a mixture of 1 volume of *formaldehyde solution* and 99 volumes of *water*. Heat at 60° for 5 minutes. Evaporate to dryness under a current of nitrogen. Dissolve the residue in 5 ml of *water* and dilute to 20.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *acetonitrile* and a buffer solution prepared by dissolving 1.32 g of *ammonium phosphate, dibasic* in 900 ml of *water*, adjusted to pH 7.0 with *orthophosphoric acid* and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the secondary peak (*trans*-4-(6,8-dibromo-1,4-dihydroquinazolin-3(2*H*)-yl)cyclohexanol) and the ambroxol peak is at least 4.0.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *water* and dilute to 50.0 ml with *water*. Dilute 5.0 ml of the solution to 50.0 ml with *water*.

Reference solution. A 0.01 per cent w/v solution of *ambroxol hydrochloride IPRS* in *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of buffer solution prepared by dissolving 1.32 g of *ammonium phosphate, dibasic* in 900 ml of *water*, adjusted to pH 7.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*, filter and *acetonitrile*,
- flow rate: 1 ml per minute,

– spectrophotometer set at 248 nm,
– injection volume: 20 µl.

The retention time of the principal peak is about 8.0 minutes.

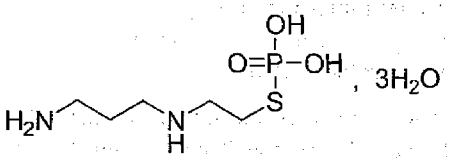
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{18}Br_2N_2O,HCl$.

Storage. Store protected from light.

Amifostine



$C_5H_{15}N_2O_3PS,3H_2O$ Mol. Wt. 268.3

Amifostine is Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester), trihydrate.

Amifostine contains not less than 78.0 per cent and not more than 82.0 per cent of $C_5H_{15}N_2O_3PS$, calculated on as-is basis.

Category. Cytoprotective agent.

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amifostine IPRS* or with the reference spectrum of amifostine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.5 to 7.5, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 150 mg of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution (a). A solution containing 0.007 per cent w/v of *amifostine thiol (ethanethiol,2-[(3-aminopropyl)amino]dihydrochloride) IPRS* and 0.0016 per cent w/v *amifostine IPRS* in *water*.

Reference solution (b). A 0.3 per cent w/v solution of *amifostine IPRS* in *water*.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Luna C8),
– sample temperature: 4°,
– mobile phase: a mixture of 72 volumes of a buffer solution prepared by dissolving 0.94 g of *sodium 1-hexanesulphonate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, and 28 volumes of *methanol*,
– flow rate: 1 ml per minute,
– spectrophotometer set at 220 nm,
– injection volume: 10 µl.

Inject reference solution (a) and (b). The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 15.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to amifostine thiol is not more than 0.64 times the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than 0.94 times the area of amifostine peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 2.8 times the area of amifostine peak in the chromatogram obtained with reference solution (a) (0.3 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Water (2.3.43). 19.2 per cent to 21.2 per cent, using Method 3, determined on 0.1 g contained in a stoppered centrifuge tube, add 10.0 ml of 4.0 per cent w/v solution of *N-ethylmaleimide* in *methanol* and sonicate for 15 minutes. Shake to disperse, and sonicate for an additional 15 minutes. Use 1.0 ml of the supernatant.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 30 mg of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution. A 0.3 per cent w/v solution of *amifostine IPRS* in *water*.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_5H_{15}N_2O_3PS$.

Storage. Store protected from light and moisture, at a temperature 2° to 8°.

Amifostine for Injection

Amifostine for Injection is a sterile, crystalline material suitable for parenteral use.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile 0.9 per cent w/v sodium chloride injection, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Amifostine for Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amifostine, $C_5H_{15}N_2O_3PS$.

The contents of the sealed containers comply with the requirements stated under Parenteral Preparations (powder for Injections) and with the following requirements.

Usual strength. 50 mg per ml.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amifostine IPRS* or with the reference spectrum of amifostine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.5 to 7.5, in a solution constituted as directed in the labelling.

Related substances.

NOTE — Prepare the solutions immediately before use.

A. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of injection containing 24 mg of amifostine in *water* and dilute to 10.0 ml with *water.*

Reference solution (a). A 0.007 per cent w/v solution of *amifostine thiol (Ethanethiol,2-[(3-aminopropyl)amino]-, dihydrochloride) IPRS* in *water.*

Reference solution (b). A solution containing 0.0015 per cent w/v of *sodium thiophosphate* and 0.0013 per cent w/v of *N,N-dimethylformamide* in *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Luna C8),
- sample temperature: 4°,
- mobile phase: a mixture of 72 volumes of a buffer solution prepared by dissolving 0.94 g of *sodium 1-hexanesulphonate* in 1000 ml of *water,* adjusted to pH 3.0 with *orthophosphoric acid* and 28 volumes of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The retention times of sodium thiophosphate and *N,N*-dimethylformamide are about 2 minutes and about 3.6 minutes, respectively.

Inject reference solution (a) and (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (a) and not more than 4.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to sodium thiophosphate is not more than 0.16 times the area of sodium thiophosphate peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any peak corresponding to *N,N*-dimethylformamide is not more than 0.16 times the area of *N,N*-dimethylformamide peak in the chromatogram obtained with reference solution (b) (0.088 per cent) and the area of any other secondary peak is not more than 0.1 per cent, calculated by area normalisation.

Inject reference solution (a) and the test solution. Calculate the percentage of amifostine thiol in the test solution.

B. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve a suitable quantity of the injection in *water* to obtain a solution containing 1.0 per cent w/v of Amifostine.

Reference solution. A 0.0046 per cent w/v solution of *amifostine disulphide(1,3-Propanediamine, N,N-(dithiodi-2,1-ethanediyl)bis, tetrahydrochloride) IPRS* in *water.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Luna C18),
- sample temperature: 4°,
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 0.4 g of *sodium 1-octanesulphonate* in 1000 ml of *water*, adjusted to pH 2.5 with *trifluoroacetic acid*, and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 247 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 4.0 per cent.

Inject the reference solution and the test solution. Calculate the percentage of amifostine disulphide in the test solution.

Total impurities, including amifostine thiol and amifostinedisulphide is not more than 2.0 per cent.

Water (2.3.43). 18.0 per cent to 22.0 per cent, using Method 3, determined on 0.1 g contained in a stoppered centrifuge tube, add 10.0 ml of 4.0 per cent w/v solution of *N-ethylmaleimide* in *methanol* and sonicate for 15 minutes. Shake to disperse, and sonicate for an additional 15 minutes. Use 1.0 ml of the supernatant.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of amifostine.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances A with the following modifications.

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve a suitable quantity of injection in *water* to obtain a solution containing 0.3 per cent w/v of Amifostine.

Reference solution. A 0.3 per cent w/v solution of *amifostine IPRS* in *water*.

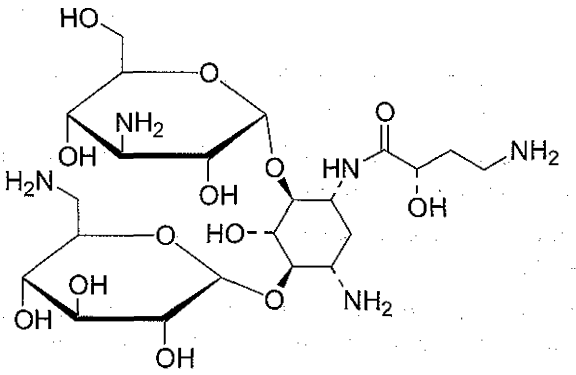
Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_5H_{15}N_2O_3PS$ in the injection.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Amikacin



$C_{22}H_{43}N_5O_{13}$ Mol. Wt. 585.6

Amikacin is (*S*)-*O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl(1→4)]-*N*^{1}-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-D-streptamine.

Amikacin contains not less than 900 µg of $C_{22}H_{43}N_5O_{13}$ per mg, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white crystalline powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 60 volumes of *methanol*, 30 volumes of *strong ammonia solution* and 25 volumes of *chloroform*.

Test solution. A 0.6 per cent w/v solution of the substance under examination.

Reference solution (a). A 0.6 per cent w/v solution of *amikacin IPRS*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 3 µl of each solution. After development, allow the plate to dry in air, heat it at 110° for 15 minutes and immediately spray it with a 1 per cent w/v solution of *ninhydrin* in a mixture of 100 volumes of 1-*butanol* and 1 volume of *pyridine*. The principal pink-coloured spot in the chromatogram obtained with the test solution corresponds to those in the chromatograms obtained with reference solution (a) and (b).

B. To 1 ml of a 1 per cent w/v solution add 1 ml of *2 M sodium hydroxide*, mix and add 2 ml of a 1 per cent w/v solution of *cobalt nitrate*; a violet colour is produced.

C. To a solution of 50 mg in 5 ml of *water* add 4 ml of a 0.035 per cent w/v solution of *anthrone* in *sulphuric acid*; a bluish-violet colour is produced.

Tests

pH (2.4.24). 9.5 to 11.5, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). + 97° to + 105°, determined in a 2.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution. A 0.01 per cent w/v solution of *amikacin IPRS* in *water*.

Derivatise the solutions prior to analysis by transferring 0.2 ml of the solution under test to a ground-glass-stoppered vial. Add 2 ml of a 1.0 per cent w/v solution of *2,4,6-trinitrobenzenesulphonic acid*. To the solution add 3 ml of *pyridine* and close the vial tightly. Shake vigorously for 30 seconds and heat in a water-bath at 75° for 45 minutes. Cool in cold water for 2 minutes and add 2 ml of *glacial acetic acid*. Shake vigorously for 30 seconds. Store the derivatised solutions at 10° prior to and during analysis.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 2),
- column temperature: 30°,
- mobile phase: a mixture of 30 volumes of a 0.27 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 6.5 with 2.2 per cent w/v solution of *potassium hydroxide* and 70 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 340 nm,
- injection volume: 20 µl.

The relative retention time with reference to amikacin for amikacin impurity A (4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-*L*-streptamine) is about 1.5.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 1.5.

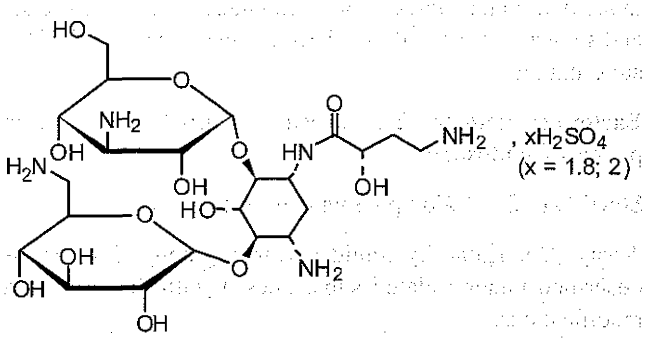
Inject the reference solution and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to amikacin impurity A is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent); the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks other than amikacin impurity A is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Sulphated ash (2.3.18). Not more than 1.0 per cent, the charred residue being moistened with 2 ml of *nitric acid* and 5 drops of *sulphuric acid*.

Water (2.3.43). Not more than 8.5 per cent, determined on 0.2 g.

Assay. Determine by the microbiological assay of antibiotics, Method B (2.2.10), and express the result in µg of Amikacin, $C_{22}H_{43}N_5O_{13}$, per mg.

Amikacin Sulphate



$C_{22}H_{43}N_5O_{13},1.8H_2SO_4$ Mol. Wt. 762.1

$C_{22}H_{43}N_5O_{13},2H_2SO_4$ Mol. Wt. 781.8

Amikacin Sulphate is (*S*)-*O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl (1→4)]-*N*^{1}-(4-amino-2-hydroxy-1-oxobutyl)- 2-deoxy-D-streptamine sulphate (1:2 or 1:1.8)(salt).

Amikacin Sulphate having a molar ratio of Amikacin to H_2SO_4 of 1:2 contains the equivalent of not less than 674 µg and not more than 786 µg of $C_{22}H_{43}N_5O_{13}$ per mg, calculated on the dried basis. Amikacin Sulphate having a molar ratio of Amikacin to H_2SO_4 of 1:1.8 contains the equivalent of not less than 691µg and not more than 806 µg of $C_{22}H_{43}N_5O_{13}$ per mg, calculated on the dried basis.

Category. Antibacterial.

Description. A white to yellowish-white crystalline powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 60 volumes of *methanol*, 30 volumes of *strong ammonia solution* and 25 volumes of *chloroform*.

Test solution. A 0.6 per cent w/v solution of the substance under examination.

Reference solution (a). A 0.6 per cent w/v solution of *amikacin IPRS*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 3 µl of each solution. After development, allow the plate to dry in air, heat it at 110° for 15 minutes and immediately spray it with a 1 per cent w/v solution of *ninhydrin* in a mixture of 100 volumes of 1-*butanol* and 1 volume of *pyridine*. The principal pink-coloured spot in the chromatogram obtained with the test solution corresponds to those in the chromatograms obtained with reference solution (a) and (b).

B. To 1 ml of a 1 per cent w/v solution add 1 ml of *2 M sodium hydroxide*, mix and add 2 ml of a 1 per cent w/v solution of *cobalt nitrate*; a violet colour is produced.

C. To a solution of 50 mg in 5 ml of *water* add 4 ml of a 0.035 per cent w/v solution of *anthrone* in *sulphuric acid*; a bluish-violet colour is produced.

Tests

pH (2.4.24). 2.0 to 4.0 (1:2 salt), or 6.0 to 7.3 (1:1.8 salt), determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). + 76.0° to + 84.0°, determined in a 2.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution. Dissolve 7.5 mg of *amikacin IPRS* in *water* and dilute to 100.0 ml with *water*.

Derivatise the solutions prior to analysis by transferring 0.2 ml of the solution under test to a ground-glass-stoppered vial. Add 2 ml of a 1.0 per cent w/v solution of *2,4,6-trinitrobenzenesulphonic acid*. To the solution add 3 ml of *pyridine* and close the vial tightly. Shake vigorously for 30 seconds and heat in a water-bath at 75° for 2 hours. Cool in cold water for 2 minutes and add 2 ml of *glacial acetic acid*. Shake vigorously for 30 seconds. Store the derivatised solutions at 10° prior to and during analysis.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 2),
- column temperature: 30°,
- mobile phase: a mixture of 30 volumes of a 0.27 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 6.5 with 2.2 per cent w/v solution of *potassium hydroxide* and 70 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 340 nm,
- injection volume: 20 µl.

The relative retention time with reference to amikacin for amikacin impurity A (4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-L-streptamine) is about 1.5.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 1.5.

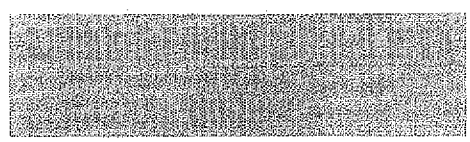
Inject the reference solution and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to amikacin impurity A is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent); the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks other than amikacin impurity A is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent). Ignore any peak eluting before the principal peak and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Sulphated ash (2.3.18). Not more than 1.0 per cent, the charred residue being moistened with 2 ml of *nitric acid* and 5 drops of *sulphuric acid*.

Loss on drying (2.4.19). Not more than 13.0 per cent, determined on 0.1 g by drying in an oven over *phosphorus pentoxide* at 110° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method B (2.2.10), and express the result in µg of amikacin, $C_{22}H_{43}N_5O_{13}$, per mg.

Labelling. The label states (1) whether the molar ratio of amikacin to H_2SO_4 of the contents is 1:2 or 1:1.8; (2) whether the material is intended for use in the manufacture of parenteral preparations.



Amikacin Injection

Amikacin Sulphate Injection

Amikacin Injection is a sterile solution of Amikacin Sulphate in Water for Injections or of Amikacin in Water for Injections prepared with the aid of Sulphuric Acid.

Amikacin Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amikacin, $C_{22}H_{43}N_5O_{13}$.

Usual strengths. The equivalent of 100 mg or 500 mg of amikacin in 2 ml.

Identification

Dilute the injection to obtain a solution containing 6 mg of amikacin per ml (test solution). The test solution complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 60 volumes of *methanol*, 30 volumes of *strong ammonia solution* and 25 volumes of *chloroform*.

Reference solution (a). A 0.6 per cent w/v solution of *amikacin IPRS*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 3 µl of each solution. After development, allow the plate to dry in air, heat it at 110° for 15 minutes and immediately spray it with a 1 per cent w/v solution of *ninhydrin* in a mixture of 100 volumes of *1-butanol* and 1 volume of *pyridine*. The principal pink-coloured spot in the chromatogram obtained with the test solution corresponds to those in the chromatograms obtained with reference solution (a) and (b).

B. To 1.5 ml of the test solution add 1 ml of *2 M sodium hydroxide*, mix and add 2 ml of a 1 per cent w/v solution of *cobalt nitrate*; a violet colour is produced.

C. To 1.5 ml of the test solution add 3.5 ml of *water* mix and add 4 ml of a 0.035 per cent w/v solution of *anthrone* in *sulphuric acid*; a bluish-violet colour is produced.

Tests

pH (2.4.24). 3.5 to 5.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing about 0.1 g of Amikacin to 10.0 ml with *water*.

Reference solution. A 0.013 per cent w/v solution of *amikacin sulphate IPRS* in *water*.

Derivatise the solutions prior to analysis by transferring 0.2 ml of the solution under test to a ground-glass-stoppered vial. Add 2 ml of a 1.0 per cent w/v solution of *2,4,6-trinitrobenzenesulphonic acid*. To the solution add 3 ml of *pyridine* and close the vial tightly. Shake vigorously for 30 seconds and heat in a water-bath at 75° for 2 hours. Cool in cold water for 2 minutes and add 2 ml of *glacial acetic acid*. Shake vigorously for 30 seconds. Store the derivatised solutions at 10° prior to and during analysis.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 2),
- mobile phase: a mixture of 30 volumes of a 0.27 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 6.5 with 2.2 per cent w/v solution of *potassium hydroxide* and 70 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 340 nm,
- injection volume: 20 µl.

The relative retention time with reference to amikacin for amikacin impurity A (4-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-*L*-streptamine) is about 1.5.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 1.5.

Inject the reference solution and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent) and the sum of areas of all the secondary peaks is not more than three times the area of the principal peak in the chromatogram obtained with the reference solution (3.0 per cent). Ignore any peak eluting before the principal peak and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

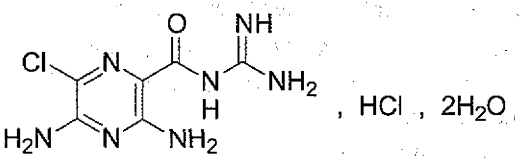
Bacterial Endotoxins (2.2.3). Not more than 0.33 Endotoxin unit per mg of amikacin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute the injection to obtain a solution containing 1 mg of amikacin per ml. Determine by the microbiological assay of antibiotics, Method B, (2.2.10) and express the result in mg of amikacin, $C_{22}H_{43}N_5O_{13}$ per ml.

Labelling. The label states the quantity of Amikacin Sulphate contained in the sealed container in terms of the equivalent amount of amikacin.

Amiloride Hydrochloride



$C_6H_8ClN_7O,HCl,2H_2O$ Mol. Wt. 302.1

Amiloride Hydrochloride is *N*-amidino-3,5-diamino-6-chloropyrazine-2-carboxamide hydrochloride dihydrate.

Amiloride Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_6H_8ClN_7O,HCl$, calculated on the anhydrous basis.

Category. Diuretic.

Description. A pale yellow to greenish-yellow powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrphotometry (2.4.6). Compare the spectrum with that obtained with *amiloride hydrochloride IPRS* or with the reference spectrum of amiloride hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel.

Mobile phase. A freshly prepared mixture of 88 volumes of *dioxan*, 6 volumes of *dilute ammonia solution* and 6 volumes of *water*.

Test solution. Dissolve 0.2 g of the substance under examination in sufficient *methanol* to produce 50.0 ml.

Reference solution. A 0.4 per cent w/v solution of *amiloride hydrochloride IPRS* in *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve 10 mg in 10 ml of *water* and add 10 ml of a 20 per cent w/v solution of *cetrimide*, 0.25 ml of *2 M sodium hydroxide* and 1 ml of *bromine water*; a greenish-yellow colour is produced. Add 2 ml of *2 M hydrochloric acid*; the solution becomes deep yellow and exhibits a blue fluorescence under ultraviolet light at 365 nm.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Free acid. Dissolve 1.0 g in 100 ml of a mixture of equal volumes of *methanol* and *water* and titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (2.4.25); not more than 0.3 ml is required.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of *water*.

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml of the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.5 volume of *tetramethylammonium hydroxide solution*, 25 volumes of *acetonitrile* and 74.5 volumes of *water*, adjusted to pH 7.0 with a mixture of 1 ml of *orthophosphoric acid* and 9 ml of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the signal–to–noise ratio of the principal peak is not less than 5.0.

Inject the reference solution and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 11.0 to 13.0 per cent, determined on 0.2 g.

Assay. Dissolve 0.25 g in a mixture of 100 ml of *anhydrous glacial acetic acid* and 15 ml of *dioxan* and add 10 ml of

mercuric acetate solution. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02661 g of $C_6H_8ClN_7O,HCl$.

Storage. Store protected from light.

Amiloride Tablets

Amiloride Hydrochloride Tablets

Amiloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O,HCl$.

Usual strength. 5 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.5 mg of anhydrous amiloride hydrochloride with 100 ml of *0.1 M hydrochloric acid* and filter. When examined in the range 230 nm to 380 nm (2.4.7), the solution shows absorption maxima at about 285 nm and 363 nm.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium: 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium, if necessary. Measure the absorbance of the resulting solution at the maximum at about 363 nm (2.4.7). Calculate the content of amiloride hydrochloride, $C_6H_8ClN_7O,HCl$ in the medium from the absorbance obtained from a solution of known concentration of *amiloride hydrochloride IPRS* in the dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_6H_8ClN_7O,HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of *water*.

Test solution. Disperse a quantity of the powdered tablets containing 17.5 mg of anhydrous Amiloride Hydrochloride with the solvent mixture and dilute to 10.0 ml with the solvent mixture, centrifuge.

Reference solution (a). A solution of *amiloride hydrochloride IPRS* containing 0.175 per cent w/v of anhydrous amiloride hydrochloride in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the reference solution (a) to 10.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (c). A 0.001 per cent w/v solution of *methyl 3,5–diamino–6–chloropyrazine–2–carboxylate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a mixture of 5 volumes of *tetramethylammonium hydroxide solution*, 250 volumes of *acetonitrile* and 745 volumes of *water*, adjusted to pH 7.0 using a mixture of 1 ml of *orthophosphoric acid* and 9 ml of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (c) and adjust the concentration of acetonitrile so that the retention time of methyl 3,5–diamino–6–chloropyrazine–2–carboxylate is 5 to 6 minutes.

Inject reference solution (a) and adjust the concentration of tetramethylammonium hydroxide and orthophosphoric acid keeping the pH at 7.0, so that the retention time of amiloride is 9 to 12 minutes.

Inject reference solution (b). The test is not valid unless the signal–to–noise ratio of the principal peak is not less than 5.0.

Inject reference solution (c) and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the secondary peaks is not more than the area of the peak due to methyl 3,5–diamino–6–chloropyrazine–2–carboxylate in the chromatogram obtained with reference solution (c) (0.6 per cent). Ignore any peak with an area less than 0.1 times the area of the peak due to methyl 3,5–diamino–6–chloropyrazine–2–carboxylate in the chromatogram obtained with reference solution (c) (0.06 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Powder one tablet and transfer to a 100-ml volumetric flask, add 60 ml of *0.1 M hydrochloric acid*, and shake by mechanical means for 30 minutes. Dilute with *0.1 M hydrochloric acid* to volume, mix, and centrifuge a portion of the mixture. Dilute a measured portion of the clear supernatant liquid quantitatively to obtain a solution containing about

10 µg of amiloride hydrochloride per ml. Measure the absorbance of the resulting solution at the maximum at about 363 nm (2.4.7).

Calculate the content of $C_6H_8ClN_7O$, HCl taking 692 as the specific absorbance at 363 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Dispese a quantity of the powder containing10 mg of anhydrous amiloride hydrochloride with 60 ml of *0.1 M hydrochloric acid*, and shake by mechanical means for 30 minutes and dilute to 100.0 ml with *0.1 M hydrochloric acid* and centrifuge a portion of the mixture. Dilute a measured portion of the clear supernatant liquid quantitatively to obtain a solution containing about 10 µg of amiloride hydrochloride per ml. Measure the absorbance of the resulting solution at the maximum at about 363 nm (2.4.7).

Calculate the content of $C_6H_8ClN_7O$,HCl taking 692 as the specific absorbance at 363 nm.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous amiloride hydrochloride.

Amiloride and Hydrochlorothiazide Tablets

Amiloride Hydrochloride and Hydrochlorothiazide Tablets

Amiloride and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O$,HCl and hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

Usual Strengths. Amiloride hydrochloride, 2.5 mg and Hydrochlorothiazide, 25 mg; Amiloride hydrochloride, 5 mg and Hydrochlorothiazide, 50 mg.

Identification

A. Disperse a quantity of the powdered tablets containing 0.1 g of hydrochlorothiazide with 50 ml of *acetone,* filter, evaporate the filtrate to dryness and dry the residue at 105° for 1 hour. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *hydrochlorothiazide IPRS* treated in the same manner or with the reference spectrum of hydrochlorothiazide.

B. In the test for Methyl 3,5-diamino-6-chloropyrazine-2-carboxylate, the principal spot in the chromatogram obtained with the test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the chromatogram obtained with reference solution (a) and (b).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). A 0.1 per cent w/v solution of *amiloride hydrochloride IPRS* in *methanol.*

Reference solution (b). A 0.1 per cent w/v solution of *hydrochlorothiazide IPRS* in *methanol.*

Reference solution (c). Dilute a suitable quantity of reference solution (a) and reference solution (b) in the dissolution medium to obtain a solution of similar concentration as the test solution.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *methanol*, 71 volumes of *water* and 4 volumes of buffer solution prepared by dissolving 13.6 g of *potassium dihydrogen orthophosphate* in 80 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 100 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 286 nm,
- injection volume: 50 µl.

Inject reference solution (c). The test is not valid unless the resolution between hydrochlorothiazide and amiloride is not less than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (c) and the test solution.

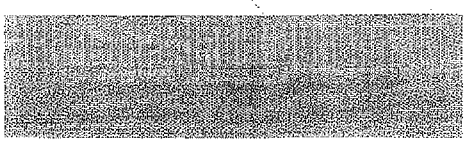
Calculate the content of $C_6H_8ClN_7O$,HCl and $C_7H_8ClN_3O_4S_2$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_6H_8ClN_7O$,HCl and not less than 75 per cent of the stated amount of $C_7H_8ClN_3O_4S_2$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254.*

Mobile phase. A mixture of 85 volumes of *ethyl acetate* and 15.0 volumes of *propan-2-ol.*

Test solution. Shake vigorously a quantity of the powdered tablets containing the 50 mg of Hydrochlorothiazide with 10 ml of *acetone* and filter.



Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *acetone.*

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air and examine under UV light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution. Disregard any spot remaining on the line of application.

Methyl 3,5-diamino-6-chloropyrazine-2-carboxylate. Determine by thin-layer chromatography (2.4.17), using a silica gel precoated plate (silica gel GF254 plates are suitable).

Mobile phase. A freshly prepared mixture of 90 volumes of *1,4 dioxane* and 12 volumes of *3M ammonia solution.*

Test solution (a). Shake vigorously a quantity of the powder containing 17.5 mg of amiloride hydrochloride with 10 ml of *methanol* and centrifuge.

Test solution(b). Dilute 1.0 ml of test solution (a) to 20.0 ml with *methanol.*

Reference solution (a). A solution containing 0.001 per cent w/v solution of *methyl 3,5-diamino-6-chloropyrazine-2-carboxylate IPRS* in *methanol.*

Reference solution(b). A solution containing 0.010 per cent w/v solution of *amiloride hydrochloride IPRS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine the spot under 365nm. Any spot corresponding to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay, using the following the solutions.

Test solution. Disperse one tablet in 2 ml of *water* with the aid of ultrasound for about 2 minutes. Add a mixture of 20 ml of *methanol* and 4 ml of *0.1 M hydrochloric acid* and sonicate for further 10 minutes and dilute to suitable volume with *water* to obtain a solution containing 0.005 per cent w/v of amiloride.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Dissolve a quantity of powder containing about 50 mg of Hydrochlorothiazide with a mixture of 20 ml of *methanol* and 4 ml of *0.1 M hydrochloric acid* sonicate for 15 minutes and dilute to 100.0 ml with *water*, mix and filter.

Reference solution (a). Dissolve a quantity of 50 mg of *hydrochlorothiazide IPRS* in a mixture of 20 ml of *methanol* and 4 ml of *0.1M hydrochloric acid* and dilute to 100.0 ml with *water.*

Reference solution (b). Dissolve 50 mg of *amiloride hydrochloride IPRS* in sufficient *methanol* to produce 200.0 ml. Dilute 20.0 ml of the resulting solution add 4 ml of *0.1 M hydrochloric acid* and dilute to 100.0 ml with *water.*

Reference solution (c). A solution contains 0.0010 per cent w/v of *4-amino-6-chlorobenzene-1,3-disulfonamide IPRS* in reference solution (a).

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, end capped, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: A mixture of 76 volumes of *water*, 20 volumes of *methanol* and 4 volumes of *phosphate buffer pH 3.0*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 286 nm,
- injection volume: 20 µl.

Inject the reference solution(c). The assay is not valid unless a peak due to 4-amino-6-chlorobenzene-1,3- disulfonamide appears immediately before the principal peak in the chromatogram obtained with reference solution (c). Increase the sensitivity, if necessary, to obtain at least 10 per cent of full-scale deflection on the chart paper for this peak. The assay is also not valid unless the height of the trough separating the two peaks is less than 10 per cent of the height of the peak due to 4-amino-6-chlorobenzene-1,3-disulfonamide. The resolution between the two peaks may be improved by decreasing the *methanol* content of the mobile phase.

Inject reference solution (a) and (b) and the test solution.

Calculate the content of $C_6H_8ClN_7O,HCl$ and $C_7H_8ClN_3O_4S_2$ in the tablets.

Storage. Store protected from light.

Labelling. The label states the strength in terms of equivalent amount of anhydrous amiloride hydrochloride and hydrochlorothiazide.

Amiloride and Frusemide Tablets

Amiloride Hydrochloride and Furosemide Tablets; Amiloride and Furosemide Tablets; Amiloride Hydrochloride and Frusemide Tablets; Frusemide and Amiloride Hydrochloride Tablets.

Amiloride Hydrochloride and Frusemide Tablets contains Amiloride Hydrochloride Dihydrate and Frusemide.

Amiloride Hydrochloride and Frusemide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the

stated amount of anhydrous amiloride hydrochloride, $C_6H_8ClN_7O$, HCl and frusemide, $C_{12}H_{11}ClN_2O_5S$.

Usual strengths. Amiloride Hydrochloride 5 mg and Frusemide 20 mg; Amiloride Hydrochloride 5 mg and Frusemide 40 mg.

Identification

A. In the Related substances, the principal spots in the chromatogram obtained with test solution (b) corresponds to the corresponding principal spot in the chromatogram obtained with reference solution (b) and (c).

B. In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the principal peaks in the chromatogram obtained with reference solution (c).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 5 volumes of *glacial acetic acid*, 5 volumes of *methanol* and 90 volumes of *chloroform*.

Test solution (a). Weigh a quantity of the powdered tablets containing 80 mg of Frusemide, add 16 ml of *methanol* and disperse with the aid of ultrasound for 5 minutes, centrifuge and use the clear supernatant liquid.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of test solution (b) to 20.0 ml with *methanol*.

Reference solution (b). A 0.05 per cent w/v solution of *frusemide IPRS* in *methanol*.

Reference solution (c). A 0.00625 per cent w/v solution of *amiloride hydrochloride IPRS* in *methanol*.

Reference solution (d). A 0.0025 per cent w/v solution of *4-chloro-5-sulfaamoylanthranilic acid IPRS* in *methanol*.

Reference solution (e). A 0.00031 per cent w/v solution of *methyl 3,5-diamino-6-chloropyazine-2-carboxylate IPRS* in *methanol*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of warm air and examine under ultraviolet light at 254 nm and 365 nm.

At 254 nm —

In the chromatogram obtained with test solution (a) any secondary spot other than any spot remaining on the line of application or any spots corresponding to either of the named impurities is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent, with reference to the content of frusemide).

At 365 nm —

Spray the plate with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed glass tank for 15 minutes (the nitrous fumes may be generated by adding *7 M sulphuric acid* dropwise to a solution containing 10 per cent w/v of *sodium nitrite* and 3 per cent w/v of *potassium iodide*). Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediaminedihydrochloride* in *ethanol (95 per cent)*. If necessary, allow to dry and repeat the spraying.

In the chromatogram obtained with test solution (a) any spot corresponding to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate is not more intense than the spot in the chromatogram obtained with reference solution (e) (0.5 per cent, with reference to the content of anhydrous amiloride hydrochloride) and any spot corresponding to 4-chloro-5-sulfamoylanthranilic acid is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.5 per cent, with reference to the content of frusemide).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 4 volumes of *water* and 6 volumes of *methanol*, adjusted to pH 2.0 with *orthophosphoric acid*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 40 mg of Frusemide in 70 ml of the solvent mixture, mix with the aid of ultrasound for 20 minutes, dilute to 100.0 ml with the solvent mixture, mix, filter. Dilute 20.0 ml of the filtrate to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.04 per cent w/v solution of *amiloride hydrochloride IPRS* in the solvent mixture.

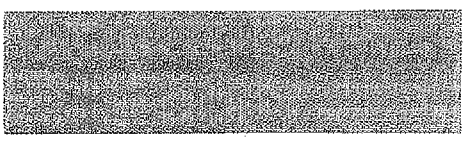
Reference solution (b). A 0.16 per cent w/v solution of *frusemide IPRS* in the solvent mixture.

Reference solution (c). Dilute reference solution (a) and (b) in the solvent mixture to obtain a solution having a known concentration similar to the test solution.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a *0.02 M* solution of *sodium hexanesulphonate* in a mixture of 40 volumes of *water* and 60 volumes of *methanol*, adjusted to pH 4.0 with *1 M acetic acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 361 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to frusemide and amiloride is not less than 2.5.



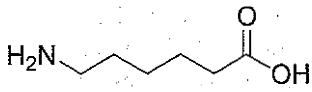
Inject reference solution (c) and the test solution.

Calculate the content of $C_6H_8ClN_7O$, HCl and $C_{12}H_{11}ClN_2O_5S$.

Storage. Store protected from light.

Labelling. The label states the strength of amiloride hydrochloride dihydrate in terms of the equivalent amount of anhydrous amiloride hydrochloride.

Aminocaproic Acid



$C_6H_{13}NO_2$ Mol. Wt. 131.2

Aminocaproic Acid is 6-aminohexanoic acid.

Aminocaproic Acid contains not less than 98.5 per cent and not more than 101.0 per cent of $C_6H_{13}NO_2$, calculated on the dried basis.

Category. Haemostatic; antifibrinolytic.

Description. Colourless crystals or a white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aminocaproic acid IPRS* or with reference spectra of aminocaproic acid.

B. Determine by thin-layer chromatography (2.4.7), coating the plate with *silica gel G*.

Mobile phase. A mixture of 25 volumes of *ethanol (95 per cent)*, 3 volumes of *water* and 4 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.25 g of the substance under examination in 100.0 ml of *water*.

Reference solution. A 0.25 per cent w/v solution of *aminocaproic acid IPRS*.

Apply to the plate 2 µl of each solution. After development, remove the plate, spray it with a 0.25 per cent w/v solution of *ninhydrin* in a mixture of equal volumes of *methanol* and *pyridine* and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 20.0 per cent w/v solution remains clear for 24 hours (2.4.1), and is colourless (2.4.1).

pH (2.4.24). 7.5 to 8.0, determined in a 20.0 per cent w/v solution.

Stability. Place 20.0 g evenly spread in a shallow dish about 9 cm in diameter, cover and allow to stand at 100° ± 2° for 72 hours. Dissolve in sufficient *water* to produce 100.0 ml. Prepare a 20.0 per cent w/v solution of the substance under examination but without the above treatment. Measure the absorbances (2.4.7) of the two solutions at the maximum at about 287 nm and at about 450 nm. Absorbance of the solution prepared from the exposed substance being examined at the maximum at about 287 nm is not more than 0.15 and of the solution of the substance under examination without the above treatment, at the maximum at about 287 nm is not more than 0.10. Absorbance of both solutions at the maximum at about 450 nm is not more than 0.03.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.2 g in about 100 ml of *anhydrous glacial acetic acid* with gentle heat to effect solution, cool. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01312 g of $C_6H_{13}NO_2$.

Aminocaproic Acid Injection

Aminocaproic Acid Injection is a sterile solution of Aminocaproic Acid in Water for Injections.

Aminocaproic Acid Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of aminocaproic acid, $C_6H_{13}NO_2$.

Usual strength. 400 mg per ml.

Identification

To a volume containing 0.4 g of Aminocaproic Acid add 2 ml of *ether*, stir, add 2 ml of *methanol*, stir again and allow to stand; the crystals after drying on a water-bath comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aminocaproic acid IPRS* or with reference spectra of aminocaproic acid.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 25 volumes of *ethanol (95 per cent)*, 3 volumes of *water* and 4 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.25 g of the substance under examination in *water* and dilute to 100.0 ml with *water*.

Reference solution. A 0.25 per cent w/v solution of *aminocaproic acid IPRS*.

Apply to the plate 2 µl of each solution. After development, remove the plate, spray it with a 0.25 per cent w/v solution of *ninhydrin* in a mixture of equal volumes of *methanol* and *pyridine* and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.0 to 7.6.

Bacterial endotoxins (2.2.3). Not more than 0.05 Endotoxin Unit per mg of aminocaproic acid.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a volume containing 0.2 g of Aminocaproic Acid, add 10 ml of *ethanol* and evaporate to dryness on a water-bath. Dissolve the residue in 100 ml of *anhydrous glacial acetic acid* by gentle heating, if necessary, cool. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01312 g of $C_6H_{13}NO_2$.

Aminocaproic Acid Tablets

Aminocaproic Acid Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aminocaproic acid, $C_6H_{13}NO_2$.

Usual strength. 500 mg.

Identification

Triturate 2 tablets with 10 ml of *water* and filter into 100 ml of *acetone*. Swirl the mixture and allow to stand for 15 minutes to complete crystallisation. Filter through a medium porosity, sintered-glass filter and wash the crystals with 25 ml of *acetone*. Apply vacuum to remove the solvent, dry at 105° for 30 minutes and cool. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aminocaproic acid IPRS* or with reference spectra of aminocaproic acid.

B. Determine by thin-layer chromatography (2.4.7), coating the plate with *silica gel G*.

Mobile phase. A mixture of 25 volumes of *ethanol (95 per cent)*, 3 volumes of *water* and 4 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.25 g of the substance under examination in *water* and dilute to 100.0 ml with *water*.

Reference solution. A 0.25 per cent w/v solution of *aminocaproic acid IPRS*.

Apply to the plate 2 µl of each solution. After development, remove the plate, spray it with a 0.25 per cent w/v solution of *ninhydrin* in a mixture of equal volumes of *methanol* and *pyridine* and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 500 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Buffer solution. A solution prepared by dissolving 13.3 g of *monobasic sodium phosphate* in 1000 ml of a solution containing 0.5 g of *sodium 1- heptane sulphonate* and 1 ml of *triethylamine*, adjusted to pH 2.2 with *orthophosphoric acid*.

NOTE — The pH of Buffer solution is critical because the diluents peak can coelute with the main peak even when the pH of Buffer solution is at 2.1 or 2.3.

Test solution. use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.1 per cent w/v solution of *aminocaproic acid IPRS* in the dissolution medium.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- sample temperature: 25°,
- mobile phase: a mixture of 25 volumes of *methanol* and 75 volumes of the buffer solution,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 25 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_6H_{13}NO_2$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_6H_{13}NO_2$.

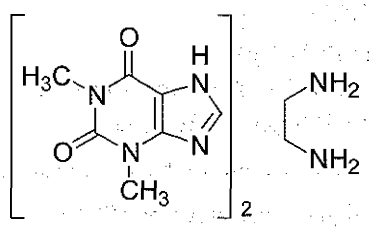
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.2 g of Aminocaproic Acid with 100 ml of *anhydrous glacial acetic acid*, heat gently to effect solution, cool. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01312 g of $C_6H_{13}NO_2$.

Aminophylline

Theophylline and Ethylenediamine



$(C_7H_8N_4O_2)_2,C_2H_8N_2$ Mol. Wt. 420.4 (anhydrous)

Aminophylline is a stable mixture or combination of theophylline and ethylenediamine. It may be anhydrous or may contain not more than two molecules of water of hydration.

Aminophylline contains the equivalent of not less than 84.0 per cent and not more than 87.4 per cent of theophylline, $C_7H_8N_4O_2$, and the equivalent of not less than 13.5 per cent and not more than 15.0 per cent of ethylenediamine, $C_2H_8N_2$, both calculated on the anhydrous basis.

Category. Bronchodilator.

Description. A white or slightly yellowish granules or powder. On exposure to air it gradually loses ethylenediamine and absorbs carbon dioxide with liberation of free theophylline. Even in the absence of light, it is gradually decomposed on exposure to a humid environment, the degradation being faster at higher temperatures.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

Dissolve 1 g in 10 ml of *water* and add 2 ml of *dilute hydrochloric acid* dropwise, with shaking. Separate the precipitate by filtration and reserve the filtrate for test D. Wash the precipitate with successive small quantities of cold *water*, recrystallise from hot *water* and dry at 100° to 105°. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *theophylline IPRS* or with the reference spectrum of theophylline.

B. To 10 mg of the residue obtained in test A add 1 ml of *hydrochloric acid* in a porcelain dish and 0.1 g of *potassium chlorate* and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of *dilute ammonia solution*; the residue acquires a purple colour. Add a few drops of *dilute sodium hydroxide solution*; the colour is discharged.

C. Saturate in *water* a portion of the residue obtained in test A and add *tannic acid solution*; a precipitate soluble in excess of the reagent is produced.

D. The filtrate complies with the following test.

To the filtrate reserved above add 0.2 ml of *benzoyl chloride*, make alkaline with *2 M sodium hydroxide* and shake vigorously. Filter, wash the precipitate with 10 ml of *water*, dissolve in 5 ml of hot *ethanol (95 per cent)* and add 5 ml of *water*. The precipitate, after washing with *water* and drying at 100° to 105° melts at 248° to 252° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *1-butanol*, 30 volumes of *acetone*, 30 volumes of *chloroform* and 10 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.2 g of the substance under examination in 2 ml of *water* with the aid of heat and dilute to 10 ml with *methanol*.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.5 per cent (for anhydrous), determined on 2.0 g dissolved in 20 ml of *pyridine*. 3.0 to 8.0 per cent (for hydrate), determined on 0.5 g.

Assay. *For theophylline* — Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *water* and 20 volumes of *methanol*.

Test solution. Dissolve 24 mg of the substance under examination in 250.0 ml with the solvent mixture.

Reference solution (a). A 0.008 per cent w/v solution of *theophylline IPRS* in the solvent mixture.

Reference solution (b). A 0.008 per cent w/v solution of theobromine in reference solution (a). Dilute 20.0 ml of the solution to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 200 ml of *methanol* and 960 mg of *sodium 1-pentanesulphonate*, diluted to 1000 ml with *water*, adjusted to pH 2.9 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

The relative retention time with respect to theophylline for theobromine is about 0.65.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to theobromine and theophylline is not less than 3.0, the tailing factor for theophylline is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of the theophylline, $C_7H_8N_4O_2$.

For ethylenediamine — Weigh 0.25 g and dissolve in 30 ml of *water*. Titrate with *0.1 M hydrochloric acid* using *methyl orange solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.003005 g of $C_2H_8N_2$.

Storage. Store protected from light and from atmospheric carbon dioxide.

Aminophylline Injection

Theophylline and Ethylenediamine Injection

Aminophylline Injection is a sterile solution of Aminophylline in Water for Injections or is a sterile solution of Theophylline in a solution of Ethylenediamine Hydrate in Water for Injections free from carbon dioxide. Aminophylline Injection may contain an excess of ethylenediamine but no other substance may be added.

Aminophylline Injection contains theophylline, $C_7H_8N_4O_2$, equivalent to not less than 73.25 per cent and not more than 88.25 per cent of the stated amount of aminophylline, and not more than 0.295 g of ethylenediamine, $C_2H_8N_2$ for each g of anhydrous theophylline, $C_7H_8N_4O_2$, determined in the Assay for theophylline.

Usual strengths. 250 mg in 10 ml; 500 mg in 20 ml.

Identification

Dilute a volume containing about 0.5 g of aminophylline with *water* to about 25 ml and add 1 ml of *dilute hydrochloric acid* with constant stirring. Separate the precipitate by filtration and reserve the filtrate for test D. Wash the precipitate with a small portion of cold *water*, recrystallise from hot *water* and dry at 100° to 105°. The crystalline powder complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *theophylline IPRS* or with the reference spectrum of theophylline.

B. To 10 mg add 1 ml of *hydrochloric acid* in a porcelain dish and 0.1 g of *potassium chlorate* and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of *dilute ammonia solution*; the residue acquires a purple colour. Add a few drops of *dilute sodium hydroxide solution*; the colour is discharged.

C. Saturate a portion in *water* and add *tannic acid solution*; a precipitate soluble in excess of the reagent is produced.

D. The filtrate complies with the following test.

Add 0.2 ml of *benzoyl chloride*, make alkaline with *2 M sodium hydroxide* and shake vigorously. Filter, wash the precipitate with 10 ml of *water*, dissolve in 5 ml of hot *ethanol (95 per cent)* and add 5 ml of *water*. The precipitate, after washing with *water* and drying at 100° to 105° melts at 248° to 252° (2.4.21).

Tests

pH (2.4.24). 8.8 to 10.0.

Bacterial endotoxins (2.2.3). Not more than 1.0 Endotoxin Unit per mg of aminophylline.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *For theophylline* — Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *water* and 20 volumes of *methanol*.

Test solution. Measure a volume containing 100 mg of theophylline to 100 ml with solvent mixture. Dilute 4.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.008 per cent w/v solution of *theophylline IPRS* in the solvent mixture.

Reference solution (b). A 0.008 per cent w/v solution of theobromine in reference solution (a). Dilute 20.0 ml of the solution to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 200 ml of *methanol* and 960 mg of *sodium 1-pentanesulfonate*, diluted to 1000 ml with *water*, adjusted to pH 2.9 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

The relative retention time with respect to theophylline for theobromine is about 0.65.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to theobromine and theophylline is not less than 3.0, the tailing factor for theophylline is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of the theophylline, $C_7H_8N_4O_2$.

For ethylenediamine — To a volume containing 0.25 g of aminophylline, add sufficient *water* to produce 30 ml. Titrate with *0.1 M hydrochloric acid* using *methyl orange solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.003005 g of $C_2H_8N_2$.

Storage. Store in single dose containers, from which carbon dioxide has been excluded. Do not allow contact with metals.

Labelling. The label states (1) the strength in terms of the equivalent amount of anhydrous aminophylline in a suitable dose-volume; (2) the route of injection; (3) that the injection is not to be used if crystals have separated.

Aminophylline Prolonged-release Tablets

Aminophylline Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Aminophylline Prolonged-release Tablets contain Aminophylline or Aminophylline Hydrate.

Aminophylline Prolonged-release Tablets contain not less than 81.4 per cent and not more than 90.0 per cent theophylline, $C_7H_8N_4O_2$ of the stated amount of aminophylline and not less than 13.5 per cent and not more than 15.0 per cent ethylenediamine, $C_2H_8N_2$ of the stated amount of aminophylline.

Usual strengths. 225 mg; 350 mg.

Identification

Disperse a quantity of the powdered tablets containing 0.5 g of Aminophylline with 20 ml of *water*, filter, add to the filtrate with constant stirring 1 ml of *2 M hydrochloric acid*, allow to stand for a few minutes and again filter. Reserve the filtrate for test C. Wash the residue with small quantities of cold *water*, recrystallise from hot *water* and dry at 105°. The residue complies with the following test.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *theophylline IPRS* or with the reference spectrum of theophylline.

B. Disperse a quantity of the powdered tablets containing 0.25 g of Aminophylline with 5 ml of *water* and filter. To 2 ml of the filtrate add 2 ml of a 1.0 per cent w/v solution of *copper (II) sulphate* and shake; a purplish blue colour is produced.

Tests

Dissolution (2.5.2). Complies with the test stated under tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. *For theophylline* —Weigh and powder 20 tablets. Disperse a quantity of the powder containing 80 mg of Aminophylline with a mixture of 20 ml of *0.1 M sodium hydroxide* and 60 ml of *water* for 10 minutes and dilute to 200.0 ml with *water* and filter. Dilute 5.0 ml of the filtrate to 250.0 ml with *0.01 M sodium hydroxide* and measure the absorbance of the solution at the maximum at 275 nm (2.4.7). Calculate the content of $C_7H_8N_4O_2$ taking 650 as the specific absorbance at the maximum at 275 nm.

For ethylenediamine—Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.3 g of Aminophylline with 20 ml of *water*, heat to 50° for 30 minutes and titrate with *0.05 M sulphuric acid*, using *bromocresol green solution* as indicator, until the colour changes from blue to green.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.003005 g of $C_2H_8N_2$.

Storage. Store protected from light and moisture.

Aminophylline Tablets

Theophylline and Ethylenediamine Tablets

Aminophylline Tablets contain theophylline, $C_7H_8N_4O_2$, equivalent to not less than 81.4 per cent and not more than 90.0 per cent of the stated amount of aminophylline, and ethylenediamine, $C_2H_8N_2$, equivalent to not less than 13.5 per cent and not more than 15.0 per cent of the stated amount of aminophylline.

Usual strength. 100 mg.

Identification

Disperse a quantity of the powdered tablets containing about 0.5 g of aminophylline with 25 ml of *water* and filter. To the filtrate add 1 ml of *dilute hydrochloric acid* with constant stirring. Separate the precipitate by filtration and reserve the filtrate. Wash the precipitate with a small portion of cold *water*, recrystallise from hot *water* and dry at 100° to 105°. The crystalline powder complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *theophylline IPRS* or with the reference spectrum of theophylline.

B. To 10 mg add 1 ml of *hydrochloric acid* in a porcelain dish and 0.1 g of *potassium chlorate* and evaporate to dryness on a water-bath; invert the dish over a vessel containing a few drops of *dilute ammonia solution*; the residue acquires a purple colour. Add a few drops of *dilute sodium hydroxide solution*; the colour is discharged.

C. Saturate a portion in *water* and add *tannic acid solution*; a precipitate soluble in excess of the reagent is produced.

The filtrate complies with the following test.

Add 0.2 ml of *benzoyl chloride*, make alkaline with *2 M sodium hydroxide* and shake vigorously. Filter, wash the precipitate with 10 ml of *water*, dissolve in 5 ml of hot *ethanol (95 per cent)* and add 5 ml of *water*. The precipitate, after washing with *water* and drying at 100° to 105° melts at 248° to 252° (2.4.21).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with *water* if necessary, at the maximum at about 269 nm (2.4.7). Calculate the content of $C_7H_8N_4O_2$ in the medium from the absorbance obtained from a known concentration of *theophylline IPRS* in the same medium.

Q. Not less than 70 per cent of the stated amount of $C_7H_8N_4O_2$.

Other tests. Comply with the tests stated under Tablets.

Assay. *For theophylline* — Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.5 g of aminophylline, transfer to a 200-ml volumetric flask with the aid of a mixture of 50 ml of *water* and 15 ml of *dilute ammonia solution* and allow to stand for 30 minutes with frequent shaking, warming to about 50°, if necessary. Cool, add *water* to volume and mix. Centrifuge the mixture, and pipette a volume of the clear supernatant liquid equivalent to about 0.25 g of aminophylline into a flask, dilute with sufficient *water* to produce 40 ml and add 8 ml of *dilute ammonia solution*. Add 20.0 ml of *0.1 M silver nitrate*, mix and boil for 15 minutes. Cool to between 5° and 10° for 20 minutes, filter at a pressure not exceeding 2.75 kPa and wash the precipitate with three quantities, each of 10 ml, of *water*. Acidify the combined filtrate and washings with *nitric acid* and add an excess of 3 ml of the acid. Cool, add 2 ml of *ferric ammonium sulphate solution*, and titrate with *0.1 M ammonium thiocyanate*.

1 ml of *0.1 M silver nitrate* is equivalent to 0.01802 g of $C_7H_8N_4O_2$.

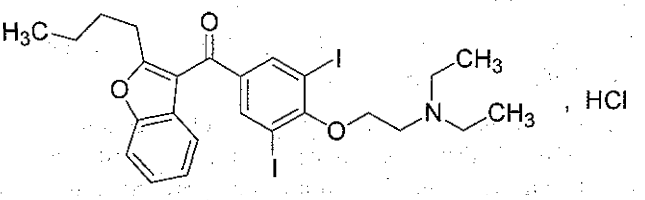
For ethylenediamine — Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.3 g of aminophylline, shake with 20 ml of *water*, heat at 50° for 30 minutes. Titrate with *0.1 M hydrochloric acid* using *methyl orange solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.003005 g of $C_2H_8N_2$.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount anhydrous aminophylline.

Amiodarone Hydrochloride



$C_{25}H_{29}I_2NO_3,HCl$ Mol. Wt. 681.8

Amiodarone Hydrochloride is 2-butylbenzofuran-3-yl-4-(2-diethylaminoethoxy)-3,5-diiodophenyl ketone hydrochloride.

Amiodarone Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{25}H_{29}I_2NO_3,HCl$, calculated on the dried basis.

Category. Antiarrhythmic.

Description. A white or almost white, fine crystalline powder.

Identification

Test B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amiodarone hydrochloride IPRS* or with the reference spectrum of amiodarone hydrochloride.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution GYS5 (2.4.1).

pH (2.4.24). 3.2 to 3.8, determined in 5.0 per cent w/v solution, prepared by dissolving in *carbon dioxide-free water* at 80° and cooling.

Impurity H. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

CAUTION—Prepare the solutions immediately before use and keep protected from bright light.

Mobile phase. A mixture of 5 volumes of *anhydrous formic acid*, 10 volumes of *methanol* and 85 volumes of *dichloromethane*.

Test solution. Dissolve 0.5 g of the substance under examination in 5.0 ml of the *dichloromethane*.

Reference solution (a). Dissolve 10 mg of *(2-chloroethyl) diethylamine hydrochloride* (amiodarone hydrochloride impurity H) in 50.0 ml of *dichloromethane*. Dilute 2.0 ml of the solution to 20.0 ml with *dichloromethane*.

Reference solution (b). Mix 2.0 ml of the test solution and 2.0 ml of reference solution (a).

Apply to the plate 50 µl of test solution, reference solution (a) and 100 µl of reference solution (b). Allow the mobile phase to rise 15 cm. Dry the plate in air and spray the plate with *potassium iodobismuthate solution* and then with *dilute hydrogen peroxide solution*, examine immediately in day light. Any spot correspond to amiodarone hydrochloride impurity H in the chromatogram obtained with the test solution is not more than the principal spot in the chromatogram obtained with reference solution (a) (0.02 per cent). The test is not valid unless the chromatogram obtained with the reference solution (b) shows clearly visible spot of amiodarone impurity H.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Dissolve 0.125 g of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution. A solution containing 0.02 per cent w/v each of *amiodarone impurity A IPRS, amiodarone impurity B IPRS* and *amiodarone hydrochloride IPRS* in *methanol*. Dilute 1.0 ml of the solution to 20.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: a mixture of 30 volumes of buffer solution prepared by diluting 3.0 ml of *glacial acetic acid* with 800 ml of *water*, adjusted to pH 4.9 with *dilute ammonia* and dilute to 1000 ml with *water*, 30 volumes of *methanol* and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Name	Relative retention time
Amiodarone impurity A[1]	0.29
Amiodarone impurity B[2]	0.37
Amiodarone	1.0

[1](2–butylbenzofuran–3–yl)(4–hydroxy–3,5–diiodophenyl) methanone,

[2](2–butylbenzofuran–3–yl)(4–hydroxyphenyl)methanone .

Inject the reference solution. The test is not valid unless the resolution between the peaks due to amiodarone impurity A and amiodarone impurity B is not less than 3.5.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). The sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Iodides. Dissolve 1.5 g in 40 ml of *water* at 80° by shaking until completely dissolved. Cool and dilute to 50.0 ml with *water* (Solution A).

To 15 ml of solution A add 1 ml of *0.1 M hydrochloric acid* and 1 ml of *0.05 M potassium iodate* and dilute to 20 ml with *water*. Allow to stand protected from light for 4 hours (Solution 1). To 15 ml of solution A add 1 ml of *0.1 M hydrochloric* acid, 1 ml of an 88.2 ppm solution of *potassium iodide* and 1 ml of *0.05 M potassium iodate* and dilute to 20 ml with *water*. Allow to stand protected from light for 4 hours (Solution 2). Measure the absorbances of solutions (1) and (2) at the maximum at about 420 nm, using as the blank a mixture of 15 ml of solution A and 1 ml of *0.1 M hydrochloric* acid diluted to 20 ml with *water* (2.4.7). The absorbance of solution (1) is not greater than half the absorbance of solution (2) (150 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 100° at a pressure not exceeding 0.3 kPa for 4 hours.

Assay. Dissolve 0.6 g in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 75 ml of *ethanol (95 per cent)*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.06818 g of $C_{25}H_{29}I_2NO_3$, HCl.

Storage. Store protected from light, at a temperature not exceeding 30°.

Amiodarone Intravenous Infusion

Amiodarone Intravenous Infusion is a sterile solution of Amiodarone Hydrochloride in Water for Injections. It is prepared immediately before use by diluting Amiodarone Sterile Concentrate with Glucose Intravenous Infusion in accordance with the manufacturer's instructions.

Amiodarone Sterile Concentrate

Amiodarone Sterile Concentrate is a sterile solution of Amiodarone Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrates for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Amiodarone Intravenous Infusion contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amiodarone hydrochloride, $C_{25}H_{29}I_2NO_3$,HCl.

Usual strengths. Concentrate, 150 mg per 3 ml ; infusion, 450 mg per 250 ml; 360 mg per 200 ml; 150 mg per 100 ml.

Identification

A. Extract a volume of the concentrate containing 0.3 g of Amiodarone Hydrochloride with three 25-ml quantities of *dichloromethane*. Dry the combined extracts over *anhydrous sodium sulphate*, filter and evaporate to dryness. To the residue, add 2 ml of *1 M sodium hydroxide* and extract with 25 ml of *ether*. Dry the extract over *anhydrous sodium sulphate*, filter and evaporate to dryness. Dry the residue obtained under reduced pressure over *phosphorus pentoxide* and dissolve in 2.5 ml of *dichloromethane*. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amiodarone hydrochloride IPRS*, treated in the same manner or with the reference spectrum of amiodarone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. The solution is not more intense than reference solution BYS4 or GYS4 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 5 volumes of *anhydrous formic acid*, 10 volumes of *methanol* and 85 volumes of *dichloromethane*.

Test solution. Dilute a volume of the concentrate containing 50 mg of Amiodarone Hydrochloride to 20.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with *methanol*.

Reference solution (b). A 0.004 per cent w/v solution of *2-butyl-3-(4-hydroxy-3,5-di-iodobenzoyl)benzofuran IPRS* in *methanol*.

Reference solution (c). A 0.1 per cent w/v solution of *benzyl alcohol* in *methanol*.

Pre-wash the plate with the mobile phase and allow it to dry in air before use.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spot corresponding to 2-butyl-3-(4-hydroxy-3,5-di-iodobenzoyl)benzofuran is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.6 per cent). Any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any spot corresponding to benzyl alcohol.

Iodides. *(NOTE- Prepare test solution (a) and (b) simultaneously and allow all of the solutions to stand protected from light for 4 hours and shake vigorously every hour).*

Solution A. To 1 g of *polysorbate 80*, add 0.2 g of *benzyl alcohol* and dilute to 10 ml with *water*.

Test solution (a). Add 1 ml of *0.1 M hydrochloric acid* to a volume of the concentrate containing 0.2 g of Amiodarone Hydrochloride, add 1 ml of *0.05 M potassium iodate* and dilute to 25.0 ml with *water*.

Test solution (b). Add 1 ml of *0.1 M hydrochloric acid* to a volume of the concentrate containing 0.2 g of Amiodarone Hydrochloride and dilute to 25 ml with *water*.

Reference solution. Use the same volume of solution A in place of the concentrate under examination and add 1 ml of *0.1 M hydrochloric acid*, 1 ml of *0.05 M potassium iodate*, 1 ml of a 0.0131 per cent w/v solution of *potassium iodide* and dilute to 25 ml with *water*.

Measure the absorbance of test solution (a) and the reference solution at 420 nm (2.4.7) using test solution (b) in the reference cell. The absorbance of the test solution is not more than the absorbance of reference solution (500 ppm).

Bacterial endotoxins (2.2.3). Not more than 8.33 Endotoxin Units per mg of amiodarone hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Infusions).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the concentrate containing 50 mg of Amiodarone Hydrochloride to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *amiodarone hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 7.5 cm x 3.9 mm, packed with silica chemically-bonded nitrile groups (4 µm) (Such as Nova-Pak CN HP),
- mobile phase: a mixture of 45 volumes of *0.01 M sodium perchlorate* and 55 volumes of *acetonitrile*, adjusted to pH 3.0 with *2 M orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 244 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{29}I_2NO_3$,HCl in the Infusion.

Storage. Store protected from light.

Labelling. The label states (1) Amiodarone Sterile Concentrate; (2) that the solution must be diluted with Glucose Intravenous Infusion.

Amiodarone Tablets

Amiodarone Hydrochloride Tablets

Amiodarone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amiodarone hydrochloride, $C_{25}H_{29}I_2NO_3$,HCl.

Usual strengths. 100 mg; 200 mg.

Identification

A. Disperse a quantity of the powdered tablets containing about 0.3 g of Amiodarone Hydrochloride with 25 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. To the residue, add 2 ml of *1 M sodium hydroxide* and extract with 25 ml of *ether*. Dry the extract over *anhydrous sodium sulphate*, filter and evaporate to dryness. Dry the residue obtained under reduced pressure over *phosphorus pentoxide* and dissolve in 2.5 ml of *dichloromethane*. The solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amiodarone hydrochloride IPRS*, treated in the same manner or with reference spectra of amiodarone hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Amiodarone Hydrochloride with 50.0 ml of *methanol*, filter. Dilute 5.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution. A solution containing 0.02 per cent w/v, each of, *amiodarone impurity A IPRS* (*(2-butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone IPRS*), *amiodarone impurity B IPRS* (*(2-butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone IPRS*) and *amiodarone hydrochloride IPRS* in *methanol*. Dilute 1.0 ml of the solution to 200.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-2),
- column temperature: 30°,
- mobile phase: a mixture of 30 volumes of *methanol*, 40 volumes of *acetonitrile* and 30 volumes of a solution prepared by adding 3 ml of *glacial acetic acid* to 800 ml of *water*, adjusted to pH 4.9 with *dilute ammonia* and dilute to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

The relative retention time with reference to amiodarone for amiodarone impurity A is about 0.29, for amiodarone impurity B is about 0.37.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to amiodarone impurity A and amiodarone impurity B is not less than 3.5.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to amiodarone impurity A is not more than the 2.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). The sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1g of Amiodarone Hydrochloride in 70 ml of *methanol* with the aid of ultrasound for 15 minutes, cool and dilute to 100.0 ml with *methanol* and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with the mobile phase.

Reference solution. Dissolve 0.1g of *amiodarone hydrochloride IPRS* in 70 ml of *methanol*, cool and dilute to 100.0 ml with *methanol*. Dilute 10.0 ml of the resulting solution to 100.0 ml with the mobile phase.

Chromatographic system

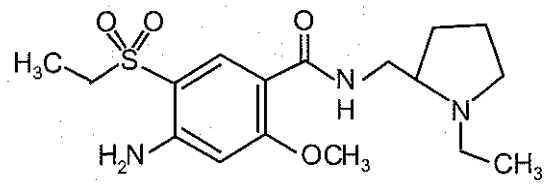
- a stainless steel column 7.5 cm x 3.9 mm, packed with very finely divided silica gel consisting of porous spherical particles with chemically bonded nitrile group (4 µm), (Such as Nova-Pack CNHP),
- mobile phase: a mixture of 45 volumes of *0.01 M sodium perchlorate* and 55 volumes of *acetonitrile*, adjusted to pH 3.0 with *2 M orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 244 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{29}I_2NO_3,HCl$ in the tablets.

Amisulpride



$C_{17}H_{27}N_3O_4S$ Mol. Wt. 369.5

Amisulpride is 4-amino-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(ethylsulphonyl)-2-methoxybenzamide.

Amisulpride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{27}N_3O_4S$, calculated on the dried basis.

Category. Antipsychotic.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amisulpride IPRS* or with the reference spectrum of amisulpride.

Tests

Appearance of solution. Dissolve 1.0 g in 3 ml of a mixture of 1 volume of *acetic acid* and 4 volumes of *water* and dilute to 20 ml with *water*. The solution is not more opalescent than OS2 (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

Optical rotation (2.4.22). – 0.10° to + 0.10°, determined in a 10.0 per cent w/v solution in *dimethylformamide*.

Impurity A. Determined by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. The upper layer obtained after shaking a mixture of 10 volumes of 50 per cent v/v solution of *ammonia*, 25 volumes of *ethanol* and 65 volumes of *di-isopropyl ether*.

Test solution. Dissolve 0.2 g of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol*.

Reference solution (a). A 0.002 per cent w/v solution of *amisulpiride impurity A IPRS (([(2RS)-1-ethylpyrrolidin-2-yl]methanamine) IPRS)* in *methanol*.

Reference solution (b). Dilute 1.0 ml of the test solution to 10.0 ml with reference solution (a).

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air, spray with *ninhydrin solution* and heat at 100° for 15 minutes. Any spot corresponding to amisulpride impurity A is not more intense

than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of mobile phase A and 70 volumes of mobile phase B.

Test solution. Dissolve 0.1 g of the substance under examination in 30 ml of *methanol* and dilute to 100.0 ml with mobile phase B.

Reference solution (a). Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 25.0 ml with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *amisulpride impurity B IPRS (4-amino-N-[[(2RS)-1-ethylpyrrolidin-2-yl]methyl]-5-(ethylsulfonyl)-2-hydroxybenzamide IPRS)* in 5.0 ml of the test solution and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *methanol*,
  B. a 0.07 per cent w/v solution of *sodium octanesulphonate* in 0.25 per cent v/v of *sulphuric acid*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	30	70
18	36	64
35	52	48
45	52	48
46	30	70
56	30	70

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to amisulpride and amisulpride impurity B is not less than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of the area of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Chlorides (2.3.12). Shake 1.25 g with 30 ml of *water* for 10 minutes and filter. The filtrate complies with the limit test of chlorides (200 ppm).

Heavy metals (2.3.13). Dissolve 4.0 g by gently heating in 5 ml of *dilute acetic acid.* Allow to cool and dilute to 20 ml with *water.* 12 ml of the resulting solution complies with the limit for heavy metals Method D (10 ppm), using 10.0 ml of *lead standard solution (2 ppm).*

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh 0.3 g and dissolve with shaking in a mixture of 5 ml of *acetic anhydride* and 50 ml of *anhydrous acetic acid.* Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03695 g of $C_{17}H_{27}N_3O_4S$.

Amisulpride Tablets

Amisulpride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amisulpride, $C_{17}H_{27}N_3O_4S$.

Usual strengths. 50 mg; 100 mg; 200 mg; 300 mg; 400 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Use the filtrate dilute, if necessary, with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_{17}H_{27}N_3O_4S$ in the medium from the absorbance obtained from a solution of known concentration of amisulpride RS, prepared by dissolving in minimum quantity of *methanol* and diluted with the dissolution medium to get similar concentration of the test solution.

Q. Not less than 70 per cent of the stated amount of $C_{17}H_{27}N_3O_4S$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of *water* and 70 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 1 g of Amisulpride with 40 ml of *water*. Add about 125 ml of *methanol* and sonicate for 30 minutes with intermittent shaking and dilute to 250.0 ml with the solvent mixture and allow to settle for 10 minutes. Dilute the solution with the solvent mixture to obtain a concentration of 0.1 per cent w/v of amisulpride, filter.

Reference solution. A 0.001 per cent w/v solution of *amisulpride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.07 per cent w/v solution of *1-octane sulphonic acid sodium* in 0.25 per cent v/v of *sulphuric acid*,
  B. *methanol*,
- a gradient programme using the condition given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile Phase A (per cent w/v)	Mobile Phase B (per cent v/v)
0	70	30
18	64	36
35	48	52
45	48	52
46	70	30
56	70	30

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of *water* and 70 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 1000 mg of Amisulpride with 40 ml of *water*. Add about 125 ml of *methanol* and sonicate for 30 minutes with intermittent shaking and dilute to 250.0 ml with the solvent mixture and allow to settle for 10 minutes. Dilute with the solvent mixture to obtain a solution having a known concentration similar to reference solution.

Reference solution. A 0.01 per cent w/v solution of *amisulpride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 90 volumes of buffer solution containing 0.07 per cent w/v solution of *1-octane sulphonic acid sodium* in 0.25 per cent v/v of *diluted sulphuric acid* and 10 volumes of *methanol*,
  B. a mixture of 10 volumes of buffer solution and 90 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile Phase A (per cent w/v)	Mobile Phase B (per cent v/v)
0	70	30
6	70	30
10	55	45
15	70	30
20	70	30

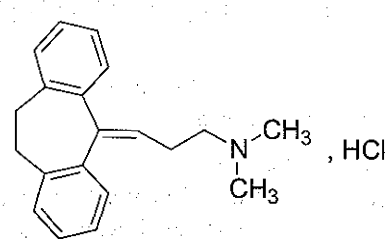
Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{27}N_3O_4S$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Amitriptyline Hydrochloride



$C_{20}H_{23}N,HCl$ Mol. Wt. 313.9

Amitriptyline Hydrochloride is 3-(10,11-dihydro-5*H*-dibenzo [*a,d*]cyclohept-5-ylidene)propyldimethylamine hydrochloride.

Amitriptyline Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{20}H_{23}N,HCl$, calculated on the dried basis.

Category. Antidepressant.

Description. Colourless crystals or a white or almost white powder.

Identification

Test A may be omitted if tests B, C, and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amitriptyline hydrochloride IPRS* or with the reference spectrum of amitriptyline hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0012 per cent w/v solution in *methanol* shows an absorption maximum only at about 239 nm, about 0.55.

C. To about 50 mg dissolved in 3 ml of *water* add 1 drop of a 2.5 per cent w/v solution of *quinhydrone* in *methanol;* no red colour is produced within 15 minutes (distinction from nortriptyline).

D. It gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. Dissolve 1.25 g in sufficient *water* to produce 25 ml. The solution is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

pH (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml of the mobile phase.

Reference solution (a). Dissolve 5.0 mg each of *amitriptyline impurity A IPRS* (*dibenzosuberone IPRS*) and *amitriptyline impurity B IPRS (cyclobenzaprine hydrochloride IPRS)* in 5.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with endcapped polar– embedded octadecylsilane bonded to amorphous organosilica polymer (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of 0.52 per cent w/v solution of *dipotassium hydrogen phosphate*, adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The relative retention time with reference to amitriptyline for amitriptyline impurity B is about 0.9 and for amitriptyline impurity A is about 2.2.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to amitriptyline impurity B and the principal peak is not less than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to amitriptyline impurity A is not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.05 per cent). The area of the peak due to amitriptyline impurity B is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in the mobile phase and dilute to 100.0 ml of the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *amitriptyline hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 30 volumes of buffer solution prepared by dissolving 1.42 g of *dibasic sodium phosphate* in 1000 ml of *water*, adjusted to pH 7.7 with *dilute orthophosphoric acid* and 70 volumes of *methanol*.

- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injection is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{23}N$,HCl.

Storage. Store protected from light.

Amitriptyline Tablets

Amitriptyline Hydrochloride Tablets

Amitriptyline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amitriptyline hydrochloride, $C_{20}H_{23}N$, HCl. The tablets are coated.

Usual strengths. 10 mg; 25 mg; 50 mg.

Identification

A. Disperse a quantity of the powdered tablets containing 20 mg of Amitriptyline Hydrochloride with 10 ml of *acetone*, filter and evaporate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amitriptyline hydrochloride IPRS* or with the reference spectrum of amitriptyline hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. The residue obtained in test A, gives reaction (A) of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 239 nm (2.4.7). Calculate the content of $C_{20}H_{23}N$, HCl in the medium from the absorbance obtained from a solution of known concentration of *amitriptyline hydrochloride IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{23}N$,HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 50 mg of Amitriptyline Hydrochloride in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A 0.0002 per cent w/v solution of *amitriptyline hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.001 per cent w/v of *amitriptyline hydrochloride IPRS*, 0.00025 per cent w/v of *amitriptyline impurity A IPRS* and 0.001 per cent w/v of *amitriptyline impurity B IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with end-capped polar-embedded octadecylsilane bonded to amorphous organosilica polymer (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 65 volumes of a 0.52 per cent w/v solution of *dipotassium hydrogen orthophosphate*, adjusted to pH 7.0 with *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Name	Relative retention time
Amitriptyline impurity B[1]	0.9
Amitriptyline Hydrochloride	1.0
Amitriptyline impurity A[2]	2.7

[1]cyclobenzaprine hydrochloride,

[2]dibenzosuberone.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to amitriptyline impurity B and amitriptyline hydrochloride is not less than 2.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak for test solution. The area of any peak corresponding to amitriptyline impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.25 per cent), the area of any peak corresponding to amitriptyline impurity B is not more than 0.2 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the

principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Powder one tablet, shake with 2.5 ml of *0.1 M hydrochloric acid* until completely disintegrated, add 5 ml of *methanol*, shake for 30 minutes, dilute the suspension to 10 ml with *methanol*, centrifuge and use the clear supernatant liquid.

Reference solution. Dissolve 25.0 mg of *amitriptyline hydrochloride IPRS* in 10 ml of *methanol* and dilute to 25.0 ml with *methanol (50 per cent)*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (10 µm),
- mobile phase: *0.03 M sodium hexanesulphonate* in a mixture of equal volumes of *acetonitrile* and *water*, adjusted to pH 4.5 with *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{23}N$,HCl in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. When tablets are film-coated, shake 20 tablets with 50 ml of *0.1 M hydrochloric acid* until completely disintegrated, add 100 ml of *methanol*, shake for 30 minutes, dilute the suspension to 200.0 ml with *methanol*, centrifuge and dilute a volume of the supernatant liquid equivalent to 25 mg of Amitriptyline Hydrochloride to 100.0 ml with *methanol (50 per cent)*.

When tablets are sugar-coated, weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Amitriptyline Hydrochloride, shake with 50 ml of *0.1 M hydrochloric acid* for 30 minutes, add 100 ml of *methanol*, shake for 30 minutes, dilute the mixture to 200.0 ml with *water*, centrifuge and use the supernatant liquid.

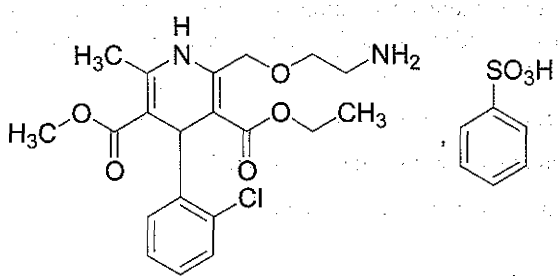
Reference solution. Dissolve 50 mg of *amitriptyline hydrochloride IPRS* in 10 ml of *methanol* and dilute to 200.0 ml with *methanol (50 per cent)*.

Use the procedure chromatographic system as described under Uniformity of content.

Calculate the content of $C_{20}H_{23}N$,HCl in the tablets.

Amlodipine Besylate

Amlodipine Besilate



$C_{26}H_{31}ClN_2O_8S$ Mol. Wt. 567.1

Amlodipine Besylate is 3-ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate.

Amlodipine Besylate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{26}H_{31}ClN_2O_8S$, calculated on the anhydrous basis.

Category. Antihypertensive; antianginal.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amlodipine besylate IPRS* or with the reference spectrum of amlodipine besylate.

B. In test A for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. When examined in the range 300 nm to 400 nm (2.4.7), a 0.005 per cent w/v solution in a 1 per cent v/v solution of *0.1 M hydrochloric acid* in *methanol* shows an absorption maximum at about 360 nm. The specific absorbance at the maximum is 113 to 121.

Tests

Optical rotation (2.4.22). −0.10° to +0.10°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. The upper layer of a mixture of 25 volumes of *glacial acetic acid*, 25 volumes of *water* and 50 volumes of *methyl isobutyl ketone*.

Test solution (a). Dissolve 0.14 g of the substance under examination in 2 ml of *methanol*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

Reference solution (a). Dissolve 70 mg of *amlodipine besylate IPRS* in 1.0 ml of *methanol*.

Reference solution (b). Dilute 0.5 ml of reference solution (a) to 5.0 ml with *methanol*.

Reference solution (c). Dilute 3.0 ml of reference solution (b) to 100.0 ml with *methanol*.

Reference solution (d). Dilute 1.0 ml of reference solution (b) to 100.0 ml with *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 80° for 15 minutes and examine under ultraviolet light at 254 nm and 365 nm. The chromatogram obtained with reference solution (a) shows two clearly separated minor spots with R_F values of about 0.18 and 0.22. In the chromatogram obtained with test solution (a) any spot, other than the spots obtained with reference solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.3 per cent) and at most 2 spots are more intense than the spot in the chromatogram obtained with reference solution (d) (0.1 per cent).

B. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b). Dilute 5.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.005 per cent w/v of *amlodipine besylate IPRS* in the mobile phase.

Reference solution (b). Dilute 3.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (c). Dissolve 5 mg of the substance under examination in 5 ml of *strong hydrogen peroxide solution*. Heat at 70° for 45 minutes.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of *methanol* and 50 volumes of a solution prepared by dissolving 7.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 10 µl.

The relative retention time between amlodipine and amlodipine impurity D (3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate) is about 0.5.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to amlodipine and amlodipine impurity D is not less than 4.5.

Inject reference solution (b), (c) and test solution (a). Run the chromatogram 3 times the retention time of the principal peak in the chromatogram obtained with test solution (a), the area of any peak due to amlodipine impurity D multiplied by 2 is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The sum of the areas of all the other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak due to benzene sulphonate (relative retention about 0.2) and any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 3.0 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and test solution (b).

Calculate the content of $C_{26}H_{31}ClN_2O_8S$.

Storage. Store protected from moisture.

Amlodipine Tablets

Amlodipine Besylate Tablets; Amlodipine Besilate Tablets

Amlodipine Tablets contain Amlodipine Besilate.

Amlodipine Tablets contain Amlodipine Besylate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amlodipine, $C_{20}H_{25}ClN_2O_5$.

Usual strengths. 5 mg; 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 500 ml of *0.01 M hydrochloric acid*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the dissolution medium if necessary, at the maximum at about 239 nm (2.4.7). Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the medium from the absorbance obtained from a solution of known concentration of *amlodipine besilate IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{25}ClN_2O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg amlodipine, dissolve in the mobile phase, dilute to 50.0 ml with the mobile phase and centrifuge.

Test solution (b). Dilute 5.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a). A solution of *amlodipine besilate IPRS* containing 0.005 per cent w/v of amlodipine in the mobile phase.

Reference solution (b). Dilute 5.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (c). Dissolve 5 mg of *amlodipine besilate IPRS* in 5 ml of strong *hydrogen peroxide solution*. Heat at 70° for 45 minutes and centrifuge.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of *methanol* and 50 volumes of a solution prepared by dissolving 7.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *phosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 10 µl.

The relative retention time between amlodipine and amlodipine impurity D (3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate) is about 0.5.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to amlodipine and impurity D is at least 4.5.

Inject test solution (a) and reference solution (b). Continue the chromatography for 3 times the retention time of amlodipine. The area of any peak corresponding to amlodipine impurity D multiplied by 2 is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of the areas of all the other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak due to benzene sulphonate (relative retention about 0.2) and any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

Test solution. Disperse one tablet in the mobile phase and dilute with the mobile phase to obtain a solution containing 0.005 per cent w/v of Amlodipine. Filter through a glass fibre filter paper.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and test solution (b).

Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of amlodipine.

Amlodipine and Atenolol Tablets

Amlodipine Besylate and Atenolol Tablets; Amlodipine Besilate and Atenolol Tablets.

Amlodipine and Atenolol Tablets contain amlodipine besylate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amlodipine, $C_{20}H_{25}ClN_2O_5$ and atenolol, $C_{14}H_{22}N_2O_3$.

Usual strengths. Amlodipine, 2.5 mg and Atenolol, 25 mg; Amlodipine, 2.5 mg and Atenolol, 50 mg; Amlodipine, 5 mg and Atenolol, 50 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.01 M hydrochloric acid*.

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and *acetonitrile*.

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution (a). A 0.112 per cent w/v solution of *atenolol IPRS* in the solvent mixture.

Reference solution (b). A 0.075 per cent w/v solution of *amlodipine besylate IPRS* in the solvent mixture.

Reference solution (c). Dilute a suitable volume of reference solution (a) and reference solution (b) with the dissolution medium to obtain a solution having similar concentration to the test solution.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with phenyl group (5 µm) (Such as Zorbax SB-Phenyl),
- sample temperature: 20°,
- mobile phase: A. a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 5.5 with *dilute sodium hydroxide* or *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
15	20	80
16	75	25
20	75	25

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for amlodipine and atenolol peaks.

Inject reference solution (c) and the test solution.

Calculate the contents of $C_{20}H_{25}ClN_2O_5$ and $C_{14}H_{22}N_2O_3$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{20}H_{25}ClN_2O_5$ and $C_{14}H_{22}N_2O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 100 mg of Atenolol in 30 ml of mobile phase A with the aid of ultrasound for 15 minutes with intermittent shaking and dilute to 50.0 ml with mobile phase A. Centrifuge the solution at 4000 rpm for 10 minutes and use the supernatant liquid.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Kromasil 100°-5-C18),
- column temperature: 30°,
- mobile phase: A. a mixture of 80 volumes of a buffer solution prepared by dissolving 3.4 g of *potassium dihydrogen phosphate*, 1.0 g of *sodium octane-1 sulphonic acid* and 0.5 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, 18 volumes of *methanol* and 2 volumes of *tetrahydrofuran*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- spectrophotometer set at 238 nm (for amlodipine) and 273 nm (for atenolol),
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Flow rate (ml per min.)
0	90	10	1.0
20	90	10	1.0
30	60	40	1.5
40	55	45	1.5
75	55	45	1.5
85	90	10	1.0
95	90	10	1.0

The relative retention time with respect to amlodipine peak for amlodipine impurity D is about 0.69.

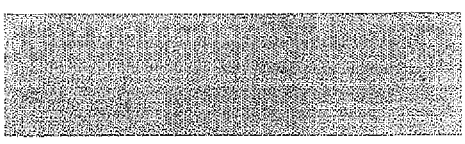
Inject the test solution and record the chromatogram at 238 nm and 273 nm.

For Atenolol —

Atenolol related impurities elutes up to 33 minutes at 273 nm, the area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalisation. Ignore any peak corresponding to amlodipine and benzene sulphonate (relative retention time at about 0.25 with respect to atenolol peak).

For Amlodipine —

Amlodipine related impurities elutes after 33 minutes at 238 nm, the area of any peak corresponding to amlodipine impurity D, multiplied with correction factor of 3.03 is not more than 0.5 per cent, the area of any other secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary



peaks, other than amlodipine impurity D is not more than 2.0 per cent, calculated by area normalisation. Ignore any peak corresponding to atenolol and benzene sulphonate (relative retention time at about 0.04 with respect to amlodipine peak).

Uniformity of content. Complies with the test stated under Tablets.

For Amlodipine — Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one intact tablet in 30 ml of the solvent mixture with the aid of ultrasound for 15 minutes with intermittent shaking, and dilute to 50.0 ml with the solvent mixture, centrifuge at 3000 rpm for 5 minutes. Dilute 5.0 ml of the supernatant liquid to 100.0 ml with *0.01 M hydrochloric acid*.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of mobile phase A and 50 volumes of *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 50 mg of Atenolol in the solvent mixture with the aid of ultrasound for 15 minutes with intermittent shaking and dilute to 100.0 ml with the solvent mixture, centrifuge at 3000 rpm for 5 minutes. Dilute 5.0 ml of the supernatant liquid to 50.0 ml with *0.01 M hydrochloric acid*.

Reference solution (a). A 0.05 per cent w/v solution of *atenolol IPRS* in the solvent mixture.

Reference solution (b). A 0.007 per cent w/v solution of *amlodipine besylate IPRS* in the solvent mixture.

Reference solution (c). Dilute a suitable volume of reference solution (a) and reference solution (b) with *0.01 M hydrochloric acid* to obtain a solution having similar concentration to the test solution.

Use chromatographic system as described under Dissolution.

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for amlodipine and atenolol peaks.

Inject reference solution (c) and the test solution.

Calculate the content of the $C_{20}H_{25}ClN_2O_5$ and $C_{14}H_{22}N_2O_3$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of amlodipine and atenolol.

Amlodipine and Benazepril Hydrochloride Capsules

Amlodipine Besylate and Benazepril Hydrochloride Capsules; Amlodipine Besilate and Benazepril Hydrochloride Capsules

Amlodipine Besylate and Benazepril Hydrochloride Capsules contain Amlodipine Besylate and Benazepril Hydrochloride equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amlodipine, $C_{20}H_{25}N_2O_5Cl$ and benazepril hydrochloride, $C_{24}H_{28}N_2O_5,HCl$.

Usual strengths. Amlodipine, 2.5 mg and Benazepril Hydrochloride, 10 mg; Amlodipine, 5 mg and Benazepril Hydrochloride, 20 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 500 ml of *0.01 M hydrochloric acid*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate and dilute if necessary, with the dissolution medium.

Reference solution (a). A 0.0385 per cent w/v solution of *amlodipine besylate IPRS* in the dissolution medium.

Reference solution (b). A 0.0225 per cent w/v solution of *benazepril hydrochloride IPRS* in the dissolution medium.

Reference solution (c). Dilute reference solution (a) and reference solution (b) in the dissolution medium to obtain a solution having similar concentration to that of the test solution.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of *methanol* and 50 volumes of a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, add 2 ml of *triethylamine* mix and adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,

- spectrophotometer set at 237 nm,
- injection volume: 50 µl.

Inject reference solution (c). The test is not valid unless the tailing factor due to amlodipine and benazepril is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{20}H_{25}N_2O_5Cl$ and $C_{24}H_{28}N_2O_5,HCl$.

Q. Not less than 80.0 per cent of the stated amounts of $C_{20}H_{25}N_2O_5Cl$ and $C_{24}H_{28}N_2O_5,HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

Solution A. Dilute 7.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and add 1.2 g of *tetrabutyl ammonium hydrogen sulphate,* Filter through a 0.45 µm nylon filter.

Solution B. Dilute 7.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid.* Filter through a 0.45 µm nylon filter.

Solvent mixture. 20 volumes of *acetonitrile*, 30 volumes of *methanol* and 50 volumes of solution B.

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing 25 mg of amlodipine and disperse in 70 ml of solvent mixture mix with the aid of ultrasound for 30 minutes, dilute to 100.0 ml with solvent mixture and filter through a 0.45 µm filter.

Reference solution (a). A solution containing 0.036 per cent w/v each of *amlodipine besylate IPRS, amlodipine impurity A IPRS* and 0.1 per cent w/v each of *benazepril hydrochloride IPRS, benazepril impurity C IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0001 per cent w/v each of *amlodipine besylate IPRS, amlodipine impurity A IPRS* and 0.0003 per cent w/v, each of, *benazepril hydrochloride IPRS, benazepril impurity C IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 80 volumes of solution A and 20 volumes of *acetonitrile*,
  B. a mixture of 80 volumes of *methanol* and 20 volumes of solution A,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 40 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
100	30	70
101	85	15
110	85	15

Name	Relative retention time
Benazepril impurity C[1]	0.23
Amlodipine impurity A[2]	0.44
Amlodipine	1.0
Benazepril	1.2

[1]{3-[1-carboxy-3-phenyl-(1*S*)-propyl]amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine}-1-acetic acid,

[2]3-ethyl 5-methyl[2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to amlodipine and benazepril is not less than 2.0 and the tailing factor is not more than 2.0 for both amlodipine and benazepril peaks.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area any peak corresponding to benazepril impurity C is not morethan 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent) and the area of any peak corresponding to amlodipine impurity A is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 12.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent). Ignore any peak with relative retention times of 0.09 and 0.10.

Other tests. Complies with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Use solution (B) and solvent mixture as described under Related substances.

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing 12.5 mg of amlodipine and transfer to a 100-ml volumetric flask, add 70 ml of solvent mixture and keep on rotary shaker for about 45 minutes and further mix sonicate with the aid of ultrasound for 30 minutes with occasional shaking and dilute to volume with solvent mixture, mix and filter, rejecting the first few ml of filtrate.

Reference solution. A solution containing 0.018 per cent w/v of *amlodipine besylate IPRS* and 0.05 per cent w/v of *benazepril hydrochloride IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile,* 30 volumes of *methanol* and 70 volumes of a buffer solution prepared by diluting 7.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and add 1.2 g of *tetrabutyl ammonium hydrogen sulphate,* Filter through a 0.45 µm nylon filter.
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}N_2O_5Cl$ and $C_{24}H_{28}N_2O_5,HCl$ in the capsules.

Storage. Store protected from light, moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of amlodipine and benazepril hydrochloride.

Amlodipine and Lisinopril Tablets

Amlodipine Besylate and Lisinopril Tablets; Amlodipine Besilate and Lisinopril Tablets

Amlodipine and Lisinopril Tablets contain amlodipine besylate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amlodipine, $C_{20}H_{25}ClN_2O_5$ and lisinopril, $C_{21}H_{31}N_3O_5$.

Usual strengths. Amlodipine, 2.5 mg and Lisinopril, 2.5 mg; Amlodipine, 5 mg and Lisinopril, 5 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid,*

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), using solvent mixture and chromatographic system as described under Assay.

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. A solution containing 0.028 per cent w/v of *amlodipine besylate IPRS* and 0.02 per cent w/v of *lisinopril IPRS* in the solvent mixture and dilute quantitatively with the dissolution medium to obtain a solution of about the same concentration as the test solution.

Inject the reference solution and the test solution.

Calculate the contents of $C_{20}H_{25}ClN_2O_5$ and $C_{21}H_{31}N_3O_5$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{25}ClN_2O_5$ and $C_{21}H_{31}N_3O_5$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse one intact tablet in 30 ml of the solvent mixture with the aid of ultrasound for 15 minutes with intermittent shaking at room temperature, dilute to 50.0 ml with the solvent mixture and filter. Further, dilute quantitatively with the solvent mixture to obtain a solution of about the same concentration as the standard solution.

Calculate the contents of $C_{20}H_{25}ClN_2O_5$ and $C_{21}H_{31}N_3O_5$ in the tablet.

Other tests. Complies with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *water* and 20 volumes of *methanol.*

Test solution. Disperse a quantity of intact tablets equivalent to 50 mg of Lisinopril in 300 ml of the solvent mixture, with the aid of ultrasound for 15 minutes with intermittent shaking at room temperature and dilute to 500.0 ml with the solvent mixture and filter. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A solution containing 0.007 per cent w/v of *amlodipine besylate IPRS* and 0.005 per cent w/v of *lisinopril IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,

- mobile phase: a mixture of 82 volumes of 0.27 per cent w/v solution of *potassium dihydrogen phosphate* and 18 volumes of *methanol*, adjusted to pH 7.0 with *triethylamine*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 212 nm,
- injection volume: 50 µl.

The elution order of the peaks is amlodipine and lisinopril.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates for amlodipine and not less than 1500 theoretical plates for lisinopril, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for both the components.

Inject the reference solution and the test solution.

Calculate the contents of $C_{20}H_{25}ClN_2O_5$ and $C_{21}H_{31}N_3O_5$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of amlodipine and lisinopril.

Amlodipine and Nebivolol Tablets

Amlodipine Besylate and Nebivolol Hydrochloride Tablets; Amlodipine Besilate and Nebivolol Hydrochloride Tablets.

Amlodipine and Nebivolol Tablets contain amlodipine besylate and nebivolol hydrochloride equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amlodipine, $C_{20}H_{25}ClN_2O_5$ and nebivolol, $C_{22}H_{25}F_2NO_4$.

Usual strength. Amlodipine, 5 mg and Nebivolol, 5 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of 1 per cent w/v solution of *sodium lauryl sulphate* in phosphate buffer pH 6.8 prepared by dissolving 6.9 g of *sodium dihydrogen phosphate* and 0.97 g of *sodium hydroxide* in about 900 ml of *water*, adjusted to pH 6.8 with *sodium hydroxide solution* or *orthophosphoric acid* and dilute to 1000 ml with *water*,

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. A solution containing *amlodipine besylate IPRS* and *nebivolol hydrochloride IPRS* equivalent to 0.055 per cent w/v, each of, amlodipine and nebivolol in *methanol* and dilute quantitatively with the dissolution medium to obtain a solution having a similar concentration as the test solution.

Use chromatographic system as described under Assay,

Inject the reference solution and the test solution.

Calculate the contents of $C_{20}H_{25}ClN_2O_5$ and $C_{22}H_{25}F_2NO_4$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{25}ClN_2O_5$ and $C_{22}H_{25}F_2NO_4$.

Related substances. Determine by liquid chromatography (2.4.14).

For Nebivolol —

Test solution. Disperse a quantity of the powdered tablets containing 20 mg of Nebivolol in 5 ml of *acetonitrile* with the aid of ultrasound for 5 minutes and dilute to 50.0 ml with the mobile phase and filter.

Reference solution. Dissolve a quantity of *nebivolol hydrochloride IPRS* containing 20 mg of nebivolol in 5 ml of *acetonitrile*, with the aid of ultrasound for 5 minutes and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile group bonded to porous silica (5 µm) (Such as Supelcosil LC-CN),
- mobile phase: a mixture of 28 volumes of *acetonitrile* and 72 volumes of a buffer solution prepared by dissolving 3.4 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, add 0.3 ml of *diethylamine*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak for test solution. The area of any secondary peak is not more than 2.5 times the area of principal peak in the chromatogram obtained with the reference solution (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (4.0 per cent). Ignore the peak due to amlodipine.

For Amlodipine —

Test solution. Disperse a quantity of the powdered tablets containing 20 mg of Amlodipine in 15 ml of the mobile phase, with the aid of ultrasound for 5 minutes and dilute to 20.0 ml with the mobile phase and filter.

Reference solution. A solution of *amlodipine besylate IPRS* containing 0.1 per cent w/v of amlodipine in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Inertsil ODS-3V),
- mobile phase: a mixture of 15 volumes of *acetonitrile,* 35 volumes of *methanol* and 50 volumes of a buffer solution prepared by mixing 7 ml of *triethylamine* in 1000 ml of *water,* adjusted to pH 3.0 with *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak for test solution. The area of any secondary peak is not more than 2.5 times the area of principal peak in the chromatogram obtained with the reference solution (2.5 per cent) and the sum of areas of all secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (4.0 per cent). Ignore the peak due to nebivolol.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse one tablet in 2 ml of *water*, add about 20 ml of the mobile phase with the aid of ultrasound for 10 minutes with intermittent shaking, and dilute to 100.0 ml with the mobile phase, filter.

Calculate the content of the $C_{20}H_{25}ClN_2O_5$ and $C_{22}H_{25}F_2NO_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 5 mg of Amlodipine in 50 ml of the mobile phase, with the aid of ultrasound for 15 minutes with intermittent shaking, and dilute to 100.0 ml with the mobile phase and filter.

Reference solution. Dissolve a suitable quantity of *amlodipine besylate IPRS* and *nebivolol hydrochloride IPRS* equivalent to amlodipine and nebivolol in the mobile phase to obtain a solution of the similar concentration as the test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile group bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 7.7 g of *sodium perchlorate* in 750 ml of *water,* add 1 ml of *triethylamine* and adjusted to pH 6.0 with *orthophosphoric acid*, and 45 volumes of *acetonitrile,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

The elution order of the peaks is amlodipine and nebivolol.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to amlodipine and nebivolol is not less than 3.0, the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for amlodipine and nebivolol peaks.

Inject the reference solution and the test solution

Calculate the content of the $C_{20}H_{25}ClN_2O_5$ and $C_{22}H_{25}F_2NO_4$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of amlodipine and nebivolol.

Amlodipine and Valsartan Tablets

Amlodipine Besylate and Valsartan Tablets; Amlodipine Besilate and Valsartan Tablets

Amlodipine and Valsartan Tablets contain amlodipine besylate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amlodipine, $C_{20}H_{25}ClN_2O_5$ and valsartan, $C_{24}H_{29}N_5O_3$.

Usual strengths. Amlodipine, 5 mg and Valsartan, 80 mg; Amlodipine, 5 mg and Valsartan, 160 mg; Amlodipine, 10 mg and Valsartan, 160 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with test solution (a) (for amlodipine) and test solution (b) (for valsartan) correspond to the principal peaks in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 1000 ml of a buffer solution prepared by dissolving 6.81 g of *monobasic potassium phosphate* and 0.9 g of *sodium hydroxide* in *water* and dilute to 1000 ml with *water*, adjusted to pH 6.8 with *0.2 M sodium hydroxide* or *1M orthophosphoric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.1 per cent w/v solution of *polysorbate 80* in the dissolution medium.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). Dissolve 40 mg, each of, *amlodipine besylate IPRS* and *valsartan IPRS* in 40 ml of *methanol* and dilute to 100.0 ml with the dissolution medium.

Reference solution (b). Dissolve 72 mg of *amlodipine besylate IPRS* in 40 ml of *methanol* and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (c). A 0.16 per cent w/v solution of *valsartan IPRS* in *methanol.*

Reference solution (d). Dilute a suitable volume of reference solution (b) and reference solution (c) with the solvent mixture to obtain a solution having known concentration similar to the test solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with phenyl group bonded to porous silica (4 µm) (Such as Synergi Polar-RP),
- column temperature: 40°,
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.2 volume of *trifluoroacetic acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject reference solution (a) and (d). The test is not valid unless the resolution between the peaks due to amlodipine and valsartan is not less than 2.0 in the chromatogram obtained with reference solution (a), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for both the peaks in the chromatogram obtained with reference solution (d).

Inject reference solution (d) and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$ and $C_{24}H_{29}N_5O_3$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{20}H_{25}ClN_2O_5$ and $C_{24}H_{29}N_5O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution (a). Disperse a quantity of intact tablets containing 50 mg of amlodipine in 50 ml of *water,* with the aid of ultrasound, add 350 ml of the solvent mixture and shake for 45 minutes. Sonicate for 15 minutes with intermittent shaking, dilute with the solvent mixture to obtain 0.02 per cent w/v of amlodipine. Centrifuge the solution for about 10 minutes at 3000 rpm. Use the clear supernatant.

Test solution (b). Dilute a suitable volume of test solution (a) to 100.0 ml with the solvent mixture to obtain a solution containing 0.016 per cent w/v of valsartan.

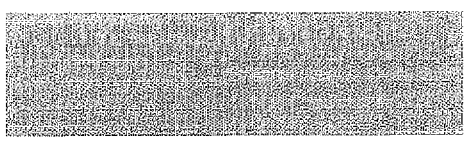
Reference solution (a). Dissolve 70 mg of *amlodipine besylate IPRS* and 80 mg of *valsartan IPRS* in 2.5 ml of *methanol* and dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (b). A 0.008 per cent w/v solution of *valsartan related compound B IPRS* in reference solution (a).

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (d). A solution containing 0.005 per cent w/v of *amlodipine related compound A IPRS* (as free base) and 0.003 per cent w/v, each of, *amlodipine besylate IPRS* and *valsartan IPRS,* prepared by dissolving in *methanol* (5 per cent of the final volume) and dilute to volume with the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 10°
- mobile phase: A. a 1 per cent v/v solution of *triethylamine* in *water,* adjusted to pH 2.8 with *orthophosphoric acid,*
  B. a mixture of 70 volumes of *methanol* and 30 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 10 µl.



Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	50	50
3	50	50
15	30	70
20	30	70
20.1	50	50
25	50	50

Name	Relative retention time
Devaleryl valsartan[1]	0.24
Amlodipine related compound A[2]	0.50
Valsartan related degradation product 1[3]	0.54
Valsartan related degradation product 2[3]	0.81
Amlodipine	1.00
Valsartan related compound B[4]	1.34
Valsartan related degradation product 3[3]	1.44
Valsartan	1.74
Valsartan related degradation product 4[3]	2.06
Valsartan ethyl ester[5]	2.32

[1]*N*-{[2′-(1*H*-Tetrazole-5-yl)biphenyl-4-yl]methyl}-*L*-valine,

[2]3-Ethyl 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate],

[3]These are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities,

[4]*N*-Butyryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-*L*-valine,

[5]*N*-Valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-*L*-valine ethyl ester.

Inject reference solution (b), (c) and (d). The test is not valid unless the resolution between the peaks due to amlodipine and valsartan related compound B and between the peaks due to valsartan related compound B and valsartan is not less than 4.0 in the chromatogram obtained with reference solution (b), the relative standard deviation for amlodipine related compound A, amlodipine and valsartan peaks is not more than 5.0 per cent in the chromatogram obtained with reference solution (d) and the signal-to- noise ratio for amlodipine and valsartan peaks is not less than 10.0 in the chromatogram obtained with reference solution (c).

Inject reference solution (d), test solution (a) and (b). In the chromatogram obtained with test solution (b), the area of any peak corresponding to devaleryl valsartan, valsartan related degradation product 1, valsartan related degradation product 2, valsartan related degradation product 3, valsartan related degradation product 4 and valsartan ethyl ester, each of, is not more than the area of the valsartan peak in the chromatogram obtained with reference solution (d) (0.2 per cent). In the chromatogram obtained with test solution (a), the area of any peak corresponding to amlodipine related compound A (free base) is not more than 1.25 times the area of amlodipine related compound A peak in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of any other secondary peak is not more than the area of amlodipine peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The sum of all the impurities is not more than 1.2 per cent. If valsartan related compound A is a potential degradation product, then sum of all the impurities other than valsartan related compound A and amlodipine related compound A is not more than 2.0 per cent. Ignore the peaks due to valsartan related compound B, benzene sulphonic acid at relative retention time about 0.19 and any peak with an area less than 0.5 times the area of the amlodipine peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Limit of valsartan related compound A. Determine by liquid chromatography (2.4.14).

NOTE — Valsartan related compound A is a process impurity and a formulation-specific degradation product.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Valsartan in the mobile phase with the aid of ultrasound and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A 0.004 per cent w/v solution, each of *valsartan related compound A IPRS* and *valsartan IPRS* in the mobile phase.

Reference solution (b). A 0.0001 per cent w/v solution of *valsartan related compound A IPRS (N*-Valeryl-*N*-{[2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl]methyl}-D-valine*)* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with cellulose tris-(3,5-dichlorophenyl carbamate bonded to porous silica (5 µm) (Such as Chiracel OD-H),
- sample temperature: 10°,
- mobile phase: a mixture of 85 volumes of *n-hexane,* 15 volumes of *2-propanol* and 0.1 volume of *trifluoroacetic acid,*
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

The relative retention time with reference to valsartan for valsartan related compound A is about 0.7.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to valsartan and valsartan related compound A is not less than 2.0 in the chromatogram obtained with reference solution (a) and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. Run the chromatogram 3.5 times the retention time of valsartan related compound A for test solution. The area of any secondary peak corresponding to valsartan related compound A is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Test solution. Disperse 1 tablet in *water* (10 per cent of the final volume) with the aid of ultrasound with intermittent shaking, dilute with the solvent mixture to obtain a solution containing 0.01 per cent w/v of Amlodipine.

Reference solution. A 0.014 per cent w/v solution of *amlodipine besylate IPRS,* prepared by adding *methanol* (upto 5 per cent of the final volume) to dissolve and dilute to volume with the solvent mixture.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent for both the peaks.

Inject reference solution (a), test solution (a) and (b).

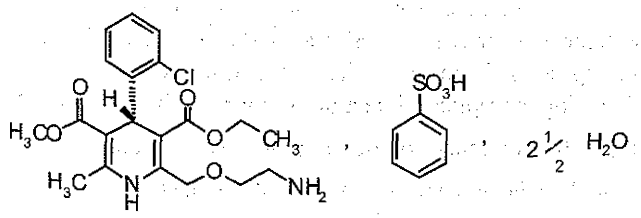
Calculate the content of $C_{20}H_{25}ClN_2O_5$ in test solution (a) and $C_{24}H_{29}N_5O_3$ in test solution (b) in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of amlodipine and valsartan.

S-Amlodipine Besylate

S-Amlodipine Besilate



$C_{26}H_{31}ClN_2O_8S, 2½ H_2O$ Mol. Wt. 612.1

S-Amlodipine Besylate is (*S*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester hemipentahydrate.

S-Amlodipine Besylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{25}ClN_2O_5,C_6H_6O_3S$, calculated on the anhydrous basis.

Category. Antihypertensive, antianginal.

Description. A white to pale yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *S-amlodipine besylate IPRS* or with the reference spectrum of *S*-amlodipine besylate.

Tests

Specific optical rotation (2.4.22). −30.0° to −24.0°, determined in a 1.0 per cent w/v solution in *methanol.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in the mobile phase and dilute to 100.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. A 0.001 per cent w/v solution of *S-amlodipine besylate IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Use the chromatographic system as described under Assay.

The relative retention time with respect to amlodipine for amlodipine impurity D (3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate) is about 0.5 and for benzene sulphonic acid is about 0.14.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to amlodipine impurity D multiplied by 2 is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak corresponding to benzene sulphonic acid.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 8.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase. Further dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.001 per cent w/v solution of *S-amlodipine besylate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Thermoquest),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by diluting 7 ml of *triethylamine* to 1000 ml with *water*, adjusted to pH 3.0 with *ortho-phosphoric acid*, 35 volumes of *methanol* and 15 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$,$C_6H_6O_3S$.

S-Amlodipine Tablets

S- Amlodipine Besilate Tablets; *S*- Amlodipine Besylate Tablets

S- Amlodipine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of *S*-amlodipine, $C_{20}H_{25}ClN_2O_5$.

Usual strengths. 1.25 mg; 2.5 mg; 5 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 500 ml of *0.01 M hydrochloric acid*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Use the filtrate, dilute if necessary, with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 239 nm (2.4.7). Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the medium from the absorbance obtained from a solution of known concentration of *S-amlodipine besylate IPRS*.

Q. Not less than 70 per cent of the stated amount of $C_{20}H_{25}ClN_2O_5$.

Uniformity of content. Complies with the test stated under tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

Test solution. Disperse 1 tablet in the mobile phase, sonicate and dilute if necessary to obtain a solution containing 0.0025 per cent w/v of S-Amlodipine in the mobile phase.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 5 mg of S- Amlodipine with the mobile phase and dilute to 200.0 ml of the mobile phase. Centrifuge 10.0 ml of the solution at 3500 rpm for 15 minutes.

Reference solution. A solution of *S-amlodipine besylate IPRS* containing 0.0025 per cent w/v of Amlodipine in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of buffer solution pH 3.0 prepared by diluting 7 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, 30 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$ in the tablets.

Labelling. The label states the strength in terms of the equivalent amount of S-Amlodipine.

Ammonium Chloride

NH_4Cl Mol. Wt. 53.5

Ammonium Chloride contains not less than 99.0 per cent and not more than 100.5 per cent of NH_4Cl, calculated on the dried basis.

Category. Expectorant; diuretic; systemic acidifier.

Description. Colourless crystals or a white, crystalline powder.

Identification

It gives the reactions of chlorides (2.3.1) and 10 ml of a 10 per cent w/v solution in *carbon dioxide-free water* gives reaction of ammonia salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 4.5 to 6.0, determined in a 5.0 per cent solution.

Arsenic (2.3.10) Dissolve 2.5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (4 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Iron (2.3.14). 2.0 g complies with the limit test for iron (20 ppm).

Calcium. To 0.2 ml of *ethanolic calcium standard solution (100 ppm Ca)* add 1 ml of a 4 per cent w/v solution of *ammonium oxalate*. After 1 minute add 1 ml of *2 M acetic acid* and 15 ml of a solution made by diluting 5 ml of a 10 per cent solution of the substance under examination with 10 ml of *water* and shake. Compare any opalescence produced with that of a standard prepared in a similar manner but using a mixture of 10 ml of *calcium standard solution (10 ppm Ca)* and 5 ml of *water* instead of the solution of the substance under examination (200 ppm).

Sulphates (2.3.17). 1.0 g complies with the limit test for sulphates (150 ppm).

Thiocyanate. Acidify 10 ml of a 10 per cent w/v solution with *hydrochloric acid* and add a few drops of *ferric chloride solution*; no red colour is produced.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

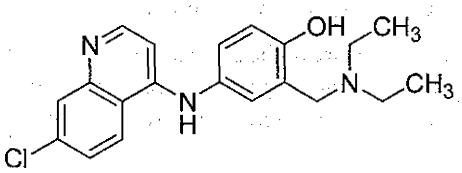
Loss on drying (2.4.19). Not more than 1.0 per cent determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.1 g in 20 ml of *water* and add a mixture of 5 ml of *formaldehyde solution*, previously neutralised to *dilute phenolphthalein solution*, and 20 ml of *water*. After 2 minutes, titrate slowly with *0.1 M sodium hydroxide* using a further 0.2 ml of *dilute phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.005349 g of NH_4Cl.

Amodiaquine Hydrochloride

Amodiaquine Dihydrochloride



, 2HCl, $2H_2O$

$C_{20}H_{22}ClN_3O$, 2HCl, $2H_2O$ Mol. Wt. 464.8

Amodiaquine Hydrochloride is 4-(7-chloro-4-quinolylamino)-2-(diethylaminomethyl)phenol dihydrochloride dihydrate.

Amodiaquine Hydrochloride contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{20}H_{22}ClN_3O$, 2HCl, calculated on the anhydrous basis.

Category. Antimalarial.

Description. A yellow, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests C and D may be omitted if tests A, B and E are carried out.

A. Dissolve 20 mg in 10 ml of *water* and add 1 ml *of strong ammonia solution*. Extract with two quantities, each of 25 ml, of *chloroform*, wash the combined *chloroform* extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate the *chloroform* and dry the residue at 105° for 2 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amodiaquine hydrochloride IPRS* treated in the same manner or with the reference spectrum of amodiaquine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 343 nm, about 0.55.

C. To 1 ml of a 2 per cent w/v solution add 0.5 ml of *cobalt thiocyanate solution*; a green precipitate is produced.

D. To 20 ml of a 2 per cent w/v solution, add 1 ml of *dilute ammonia solution*. Shake and filter; the filtrate gives the reactions of chlorides (2.3.1).

E. The undried material melts at about 158° (2.4.21).

Tests

pH (2.4.24). 3.6 to 4.6, determined in a 2.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *chloroform* saturated with *strong ammonia solution* and 10 volumes of *ethanol*.

Test solution. Add to 0.2 g of the substance under examination in a glass-stoppered test-tube 10 ml of *chloroform* saturated with *strong ammonia solution*, shake vigorously for 2 minutes, allow the solids to settle and decant the supernatant liquid.

Reference solution (a). Prepare in the same manner as the test solution but using 0.2 g of *amodiaquine hydrochloride IPRS* and 10 ml of *chloroform* saturated with *strong ammonia solution*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) with sufficient *chloroform* saturated with *strong ammonia solution* to obtain 200.0 ml.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and no secondary spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 6.0 to 10.0 per cent, determined on 0.5 g.

Assay. Dissolve 0.3 g in sufficient *0.1 M hydrochloric acid* to produce 200.0 ml. Dilute 10.0 ml of the solution to 1000.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 343 nm (2.4.7), using *0.1 M hydrochloric acid* as the blank.

Calculate the content of $C_{20}H_{22}ClN_3O$, 2HCl from the absorbance obtained by carrying out the Assay simultaneously on *amodiaquine hydrochloride IPRS*.

Amodiaquine Tablets

Amodiaquine Hydrochloride Tablets

Amodiaquine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of amodiaquine, $C_{20}H_{22}ClN_3O$.

Usual strength. The equivalent of 200 mg of amodiaquine. (1 g of Amodiaquine Hydrochloride anhydrous is approximately equivalent to 0.83 g of amodiaquine).

Identification

A. Extract the powdered tablets with *water* and filter. To 1 ml of the filtrate add 0.5 ml of *cobalt thiocyanate solution*; a green precipitate is produced.

B. The powdered tablets give the reactions of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with *water* if necessary, at the maximum at about 343 nm (2.4.7). Calculate the content of $C_{20}H_{22}ClN_3O$ in the medium from the absorbance obtained from a known concentration of *amodiaquine hydrochloride IPRS* in the same medium.

Q. Not less than 70 per cent of the stated amount of $C_{20}H_{22}ClN_3O$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *chloroform* saturated with *strong ammonia solution* and 10 volumes of *ethanol*.

Test solution. Disperse a quantity of the powdered tablets containing 40 mg of Amodiaquine Hydrochloride with 20 ml of *water* for 1 minute, add 25 ml of *chloroform* and 1 ml of *strong ammonia solution* and shake vigorously for 2 minutes. Filter the chloroform extract through a cotton plug previously soaked in *chloroform*, evaporate the filtrate to dryness and dissolve the residue in 2 ml of *chloroform* saturated with *strong ammonia solution*.

Reference solution (a). Prepare in the same manner as the test solution but using 0.2 g of *amodiaquine hydrochloride IPRS* and 10 ml of *chloroform* saturated with *strong ammonia solution*

Reference solution (b). Dilute 1.0 ml of reference solution (a) with sufficient *chloroform* saturated with *strong ammonia solution* to obtain 200.0 ml.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and no secondary spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with reference solution (b).

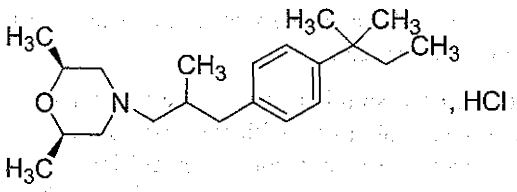
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.3 g of amodiaquine, add 100 ml of *0.1 M hydrochloric acid* and heat on a water-bath for about 15 minutes with occasional stirring. Cool, transfer to a 200-ml graduated flask and dilute to volume with *0.1 M hydrochloric acid*. To 10.0 ml of the clear supernatant liquid in a separator, add 10 ml of *0.1 M hydrochloric acid* and extract with 20 ml of *chloroform*. Discard the chloroform extract. Add 4.5 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml of *chloroform*. Extract the combined chloroform solutions with three quantities, each of 50 ml, of *0.1 M hydrochloric acid* and dilute with sufficient *0.1 M hydrochloric acid* to produce 200.0 ml. Dilute 10.0 ml with sufficient *0.1 M hydrochloric acid* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 343 nm (2.4.7), using *0.1 M hydrochloric acid* as the blank.

Calculate the content of $C_{20}H_{22}ClN_3O$, 2HCl from the absorbance obtained by carrying out the Assay simultaneously on *amodiaquine hydrochloride IPRS*. Multiply the result by 0.830 to get the equivalent quantity of $C_{20}H_{22}ClN_3O$.

Labelling. The label states the strength in terms of the equivalent amount of amodiaquine.

Amorolfine Hydrochloride



$C_{21}H_{35}NO$, HCl Mol. Wt. 354.0

Amorolfine Hydrochloride is (2*R**,6*S**)-2,6-Dimethyl-4-{2-methyl-3-[4-(2-methylbutan-2-yl)phenyl]propyl}morpholine.

Amorolfine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{35}NO$, HCl, calculated on the dried basis.

Category. Antifungal.

Description. A white to off-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amorolfine hydrochloride IPRS* or with the reference spectrum of amorolfine hydrochloride.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, 30 volumes of *acetonitrile* and 30 volumes of *tetrahydrofuran*, adjusted to pH 6.5 with *1 M sodium hydroxide*,
- flow rate: 2 ml per minute.
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Inject the test solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 1.0 per cent and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

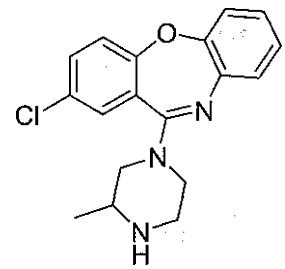
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.2 g in 50 ml of *1.0 per cent w/v mercuric acetate solution* in *glacial acetic acid*. Titrate with *0.1M perchloric acid* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0354 g of $C_{21}H_{35}NO$, HCl.

Calculate the content of $C_{21}H_{35}NO$, HCl.

Amoxapine



$C_{17}H_{16}ClN_3O$ Mol. Wt. 313.8

Amoxapine is Dibenz [b,f] [1,4] oxazepine, 2-chloro-11-(1-piperazinyl)-; 2-chloro-11-(1-piperazinyl) dibenz[b,f] [1,4] oxazepine.

Amoxapine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{16}ClN_3O$, calculated on the dried basis.

Category. Antidepressant.

Description. A white to yellowish crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained from *amoxapine IPRS* or with the reference spectrum of amoxapine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 3.9 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 7.3 with *acetic acid* or *dilute ammonia*.

Solvent mixture. 30 volumes of buffer solution and 70 volumes of *acetonitrile*.

Test solution. Dissolve 100 mg of the substance under examination in solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.1 per cent w/v of *amoxapine IPRS* and 0.00015 per cent w/v of *amoxapine related compound G IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0001 per cent w/v of *amoxapine IPRS* and 0.00015 per cent w/v, each of, *amoxapine related compound G IPRS* and *amoxapine related compound D IPRS* in the solvent mixture.

Use the chromatographic system as described under Assay except gradient programme.

Time (in min.)	Buffer solution (per cent v/v)	Acetonitrile (per cent v/v)
0	70	30
5	70	30
7.5	60	40
15	60	40
20	20	80
25	20	80
30	70	30
35	70	30

Name	Relative retention time
Chlorophenoxyanilineurea analog[1]	0.57
Amoxapine	1.0
Amoxapine related compound G[2]	1.4
Amoxapine related compound D[3]	1.7
Chlorophenoxyaniline[4]	2.9
Chlorophenoxyaniline carbamate[5]	3.8
N-Carbamoyl amoxapine[6]	4.3
Amoxapine dimer[7]	5.0

[1]N-[2-(4-Chlorophenoxy) phenyl] piperazine-1-carboxamide,

[2]3-chloro-11-(piperazin-1-yl) dibenzo [b,f][1,4] oxazepine.

[3]*2-chlorodibenzo {b,f}-[1,4]-oxazepin-11-one.*

[4]2-(4-Chlorophenoxy) aniline,

[5]Ethyl [2-(4-Chlorophenoxy) phenyl] carbamate,

[6]4-(2-Chlorodibenzo {b,f} [1,4] oxazepin-11-yl)-N-[2-(4-chlorophenoxy) phenyl] piperazine-1-carboxamide,

[7]1,4-Bis(2-chlorodibenzo[b,f] [1,4] oxazepine-11-yl) piperazine.

Inject reference solution (a). The peak to valley ratio between the peaks due to amoxapine and amoxapine related compound G is not less than 3.

Inject reference solution (b). The test is not valid unless the relative standard deviation for amoxapine, amoxapine related compound G and amoxapine related compound D peaks is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to chlorophenoxyaniline urea analog, chlorophenoxyaniline, chlorophenoxyaniline carbamate, N-carbamoyl amoxapine, amoxapine dimer and any other secondary peak is not more than the area of the principal peak due to amoxapine in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any peak due to amoxapine related compound G and D is not more than the area of the principal peak due to amoxapine related compound G and D respectively in the chromatogram obtained with reference solution (b) (0.15 per cent). The sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak due to amoxapine in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak due to amoxapine in the chromatogram obtained with reference solution (b) (0.02 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 3.9 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 7.3 with *acetic acid or dilute ammonia solution*.

Solvent mixture. 30 volumes of buffer solution and 70 volumes of *acetonitrile*.

Test solution. Dissolve 10 mg of the substance under examination in 50 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.01 per cent w/v of *amoxapine IPRS* and *amoxapine related compound G IPRS [3-chloro-11-(piperazin-1-yl) dibenzo [b,f] [1,4] oxazepine]* in the solvent mixture.

Reference solution (b). A 0.01 per cent w/v solution of *amoxapine IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 7.5 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (2.7 µm),
- column temperature: 35°,
- mobile phase: a mixture of 70 volumes of a buffer solution and 30 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Name	Relative retention time
Amoxapine	1.0
Amoxapine related compound G	1.3

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to amoxapine and amoxapine related compound G is not less than 1.5 in the chromatogram obtained with reference solution (a). The tailing factor is between 0.8 to 1.8 and the relative standard deviation for replicate injections is not more than 0.73 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_{17}H_{16}ClN_3O$.

Storage. Store protected from moisture.

Amoxapine Tablets

Amoxapine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amoxapine, $C_{17}H_{16}ClN_3O$.

Usual strengths. 25 mg; 50 mg; 100 mg; 150 mg.

Identification

A. Triturate a quantity of powered tablets containing 50 mg of Amoxapine with 10 ml of *chloroform* and filter. Evaporate to dryness on water bath. The residue complies with the following test. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxapine IPRS* or with the reference spectrum of amoxapine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of gastric juice, artificial (without pepsin) prepared by dissolving 2.0 g of *sodium chloride* in 80 ml of *1 M hydrochloric acid* and dilute to 1000 ml with *water*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the dissolution medium and filter. Measure the absorbance of the filtrate, suitably diluted with dissolution medium if necessary, at the maximum at about 294 nm (2.4.7). Calculate the content of $C_{17}H_{16}ClN_3O$ in the medium from the absorbance obtained by repeating the determination using a solution of known concentration of *amoxapine IPRS* in dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{16}ClN_3O$.

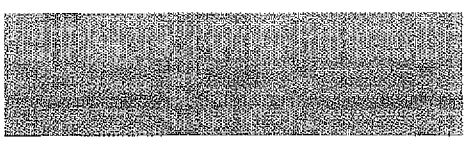
Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of *amoxapine* add 25 ml of mobile phase and shake vigorously with the aid of ultrasound for 20 minutes and dilute to 50.0 ml with the mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve a quantity of *amoxapine IPRS* in the *acetonitrile* to obtain a solution containing 0.1 per cent w/v of amoxapine. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecyl silane bonded to porous silica (5 µm),
- mobile phase: mix 20 ml of 11.3 per cent w/v of *tetramethylammonium chloride* with 4.0 ml of *dilute orthophosphoric acid* (1 in 5), and 720 ml of *acetonitrile* diluted to 2000 ml with 0.138 per cent w/v of *monobasic sodium phosphate*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.



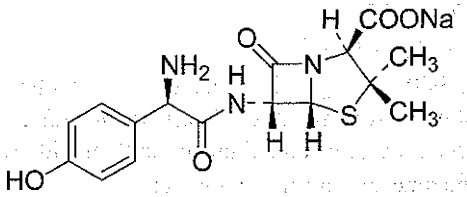
Inject the reference solution. The test is not valid unless the column efficiency is not less than 1200 theoretical plates, the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{16}ClN_3O$.

Storage. Store protected from moisture.

Amoxycillin Sodium



$C_{16}H_{18}N_3NaO_5S$ Mol. Wt. 387.4

Amoxycillin Sodium is sodium (6*R*)-6-(α-4-hydroxyphenyl-D-glycylamino)penicillanate.

Amoxycillin Sodium contains not less than 85.0 per cent and not more than 100.5 per cent of $C_{16}H_{18}N_3NaO_5S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white powder; very hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin sodium IPRS* or with the reference spectrum of amoxycillin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. A 5.0 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1) when examined immediately after preparation. The solution may initially show a pink colour and its absorbance after 5 minutes at about 430 nm is not more than 0.20 (2.4.7).

pH (2.4.24). 8.0 to 10.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +240° to +290°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate*.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method A.

Sodium chloride. Not more than 2.0 per cent, calculated on the anhydrous basis, determined by the following method. Dissolve 1.0 g in 50 ml of *water*; add 10 ml of *2 M nitric acid* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25) using a silver indicator electrode and a mercury-mercurous sulphate reference electrode or any other suitable electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005845 g of NaCl.

2-Ethylhexanoic acid (2.3.51). Not more than 0.8 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 4.0 per cent, determined on 0.4 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjusted to pH 5.0 with 4.5 per cent w/v solution of *potassium hydroxide*.

Test solution. Dissolve a quantity containing 120 mg of Amoxycillin in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Use the solution within 6 hours.

Reference solution. Dissolve a suitable quantity of *amoxycillin trihydrate IPRS* in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use the solution within 6 hours.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{18}N_3NaO_5S$ by multiplying the content of $C_{16}H_{19}N_3O_5S$ by 1.06.

Amoxycillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate

procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per mg of amoxycillin.

Amoxycillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture, at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Amoxycillin Injection

Amoxicillin Sodium Injection; Amoxycillin Sodium Injection

Amoxycillin Injection is a sterile material consisting of Amoxycillin Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Amoxycillin Injection contains Amoxycillin Sodium equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$.

Usual strengths. The equivalent of 100 mg, 250 mg, 500 mg and 1 g of amoxycillin.

Description. A white or almost white powder; very hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin sodium IPRS* or with the reference spectrum of amoxycillin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. A 5.0 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1) when examined immediately after preparation. The solution may initially show a pink colour and its absorbance after 5 minutes at about 430 nm is not more than 0.20 (2.4.7).

pH (2.4.24). 8.0 to 10.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +240° to +290°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate*.

N,N-Dimethylaniline (2.3.21). Not more than 20 ppm, determined by Method A.

Sodium chloride. Not more than 2.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh 1.0 g, dissolve in 50 ml of *distilled water*; add 10 ml of *2 M nitric acid* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25) using a silver indicator electrode and a mercury-mercurous sulphate reference electrode or any other suitable electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005845 g of NaCl.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per mg of amoxycillin.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

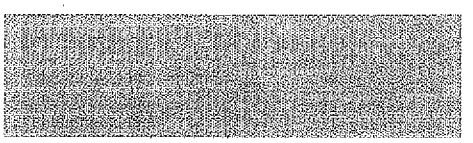
Water (2.3.43). Not more than 4.0 per cent, determined on 0.4 g.

Other tests. Comply with the tests stated under Parenteral Preparations (Injection).

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjusted to pH 5.0 with a 4.5 per cent w/v solution of *potassium hydroxide*.

Test solution. Determine the weight of the contents of 10 containers. Transfer a weighed quantity of the mixed contents of the 10 containers containing 100 mg of amoxycillin to a 100-ml volumetric flask, add 80 ml of the solvent mixture and dissolve by shaking and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use the solution within 6 hours.



Reference solution. Dissolve a quantity of *amoxycillin trihydrate IPRS* in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use the solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

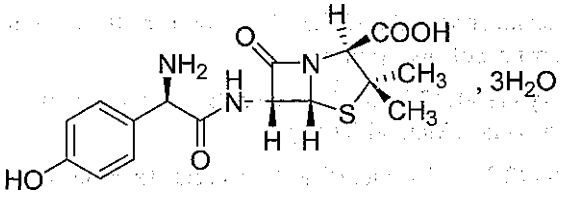
Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_5S$ in the injection.

Storage. Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°.

Labelling. The label states the quantity of Amoxycillin Sodium contained in the sealed container in terms of the equivalent amount of amoxycillin.

Amoxycillin Trihydrate



$C_{16}H_{19}N_3O_5S,3H_2O$ Mol. Wt. 419.4

Amoxycillin Trihydrate is (6*R*)-6-(α-4-hydroxyphenyl-D-glycylamino)penicillanic acid trihydrate.

Amoxycillin Trihydrate contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{16}H_{19}N_3O_5S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin trihydrate IPRS* or with the reference spectrum of amoxycillin trihydrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Place about 2 mg in a test-tube. Moisten with 0.05 ml of *water* and add 2 ml of *sulphuric acid-formaldehyde reagent*. Mix the contents of the tube by swirling; the solution is practically colourless. Place the tube in a water-bath for 1 minute; a dark yellow colour develops.

Tests

Appearance of solution. Dissolve 1.0 g in 10 ml of *0.5 M hydrochloric acid*, and a further 1.0 g in a mixture of 3 ml of *dilute ammonia solution* and 7 ml of *water*. Both solutions when freshly prepared are not more opalescent than opalescence standard OS2 (2.4.1).

pH (2.4.24). 3.5 to 5.5, determined in a 0.2 per cent w/v solution.

Specific optical rotation (2.4.22). +290° to +315°, determined in a 0.2 per cent w/v solution in *carbon dioxide-free water*.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method A.

NOTE —Test to be performed only if N,N Dimethylaniline is used in the synthesis.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Water (2.3.43). 11.5 to 14.5 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjusted to pH 4.5 with a 4.5 per cent w/v solution of *potassium hydroxide*.

Test solution. Transfer a weighed quantity of about 0.12 g of the substance under examination to a 100-ml volumetric flask, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Use the solution within 6 hours.

Reference solution. Weigh a suitable quantity of *amoxycillin trihydrate IPRS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use the solution within 6 hours.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_5S$.

Storage. Store at a temperature not exceeding 30°.

Amoxycillin Capsules

Amoxycillin Trihydrate Capsules; Amoxicillin Trihydrate Capsules; Amoxicillin Capsules

Amoxycillin Capsules contain Amoxycillin Trihydrate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$.

Usual strengths. The equivalent of 250 mg and 500 mg of amoxycillin.

Identification

Disperse a quantity of the contents of the capsules containing about 0.5 g of amoxycillin with 5 ml of *water* for 5 minutes, filter, wash the residue first with *ethanol* and then with *ether* and dry at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin trihydrate IPRS* or with the reference spectrum of amoxycillin trihydrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 60 minutes.

Use one capsule in the vessel for each test.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 272 nm (2.4.7). Similarly measure the absorbance of a standard solution of known concentration of *amoxicillin trihydrate IPRS* at about 272 nm and calculate the content of $C_{16}H_{19}N_3O_5S$.

Q. Not less than 80 per cent of the stated amount of $C_{16}H_{19}N_3O_5S$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjusted to pH 5.0 with a 4.5 per cent w/v solution of *potassium hydroxide*.

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing 0.1 g of amoxicillin, add about 80 ml of the solvent mixture and dissolve by shaking for 15 minutes and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use the solution within 6 hours.

Reference solution. Dissolve a suitable quantity of *amoxycillin trihydrate IPRS* in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 1.2 mg per ml. Use the solution within 6 hours.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_5S$ in the capsules.

Storage. Store protected from moisture.

Labelling. The label states the quantity of the active ingredient in terms of the equivalent amount of amoxycillin.

Amoxycillin Dispersible Tablets

Amoxycillin Trihydrate Dispersible Tablets; Dispersible Amoxicillin Tablets

Amoxycillin Dispersible Tablets contain Amoxycillin Trihydrate in a suitable dispersible base.

Amoxycillin Dispersible Tablets contain Amoxycillin Trihydrate equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$.

Usual strengths. The equivalent of 125 mg and 250 mg of amoxycillin.

Identification

Disperse a quantity of the powdered tablets containing about 0.5 g of amoxycillin with 5 ml of *water* for 5 minutes, filter, wash the residue first with *ethanol* and then with *ether* and dry for 1 hour at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amoxycillin trihydrate IPRS* or with the reference spectrum of amoxycillin trihydrate.

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjusted to pH about 4.5 with a 4.5 per cent w/v solution of *potassium hydroxide*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of amoxicillin and dissolve in the solvent mixture by shaking for 15 minutes and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use the solution within 6 hours.

Reference solution. Weigh a suitable quantity of *amoxycillin trihydrate IPRS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 0.12 per cent. Use the solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_5S$ in the tablets.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) the strength in terms of the equivalent amount of amoxycillin; (2) that the tablets should be dispersed in water immediately before use.

Amoxycillin Oral Suspension

Amoxicillin Oral Suspension

Amoxycillin Oral Suspension is a mixture consisting of Amoxycillin Trihydrate with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of *Water* just before use.

Amoxycillin Oral Suspension contains Amoxycillin Trihydrate equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxicillin $C_{16}H_{19}N_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of amoxycillin $C_{16}H_{19}N_3O_5S$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Usual strengths. Amoxycillin 125 mg per 5 ml; Amoxycillin 250 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Tests

pH (2.4.24). 5.0 to 7.5.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjusted to pH 4.5 with a 4.5 per cent w/v solution of *potassium hydroxide*.

Test solution. Transfer a quantity containing 0.1 g of amoxicillin to a 100-ml volumetric flask, dissolve in the solvent mixture and dilute to 100.0 ml with the solvent mixture and filter. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. Dissolve a quantity of *amoxycillin trihydrate IPRS* in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to obtain a solution having a known concentration of about 0.2 mg per ml of amoxycillin. Use the solution within 6 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 4 volumes of *acetonitrile* and 96 volumes of the solvent mixture,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1700 theoretical plates, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{16}H_{19}N_3O_5S$ weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Labelling. The label states (1) the quantity of active ingredient in terms of the equivalent amount of amoxicillin; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

Amoxycillin and Potassium Clavulanate Injection

Amoxicillin and Potassium Clavulanate injection

Amoxycillin and Potassium Clavulanate injection is a sterile material consisting of Amoxycillin Sodium and Potassium Clavulanate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Amoxycillin and Potassium Clavulanate Injection contains not less than 90.0 per cent and not more than 107.5 per cent of the stated amounts of amoxycillin, $C_{16}H_{19}N_3O_5S$ and of clavulanic acid, $C_8H_9NO_5$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strengths. 0.3 g per 10 ml; 0.6 g per 10 ml; 1.2 g per 10 ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254* (such as Merck silica gel 60 GF254 plates).

Mobile phase. A mixture of 1 volume of *butan-1-ol*, 2 volumes of a 0.1 per cent w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate*.

Test solution. Disperse a quantity of the contents of the sealed container containing about 0.4 g of clavulanic acid in 100 ml of a mixture of 4 volumes of *methanol* and 6 volumes of *0.1 M mixed phosphate buffer pH 7.0* and filter.

Reference solution. A solution containing 0.4 per cent w/v of *lithium clavulanate IPRS* and 0.8 per cent w/v of *amoxycillin trihydrate IPRS* in a mixture of 4 volumes of *methanol* and 6 volumes of *0.1 M mixed phosphate buffer pH 7.0*.

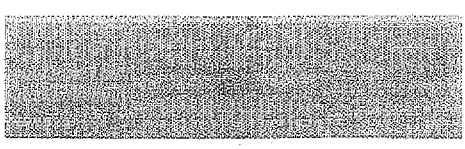
Apply to the plate 1 µl of each of the solutions after impregnating the plate by spraying it with a 0.1 per cent w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allowing to dry overnight and activating the plate by heating at 105° for 1 hour just before use. After development, allow it to dry in air and examine under ultraviolet light at 254 nm. The principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the Assay, the retention time of the two principal peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 8.0 to 10.0, determined in a solution containing about 10 per cent w/v of amoxycillin.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per mg of amoxycillin.



Water (2.3.43). Not more than 3.5 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Determine the weight of the contents of 10 containers. Dissolve, with shaking, a quantity of the mixed contents of the 10 containers containing about 60 mg of amoxycillin in *water* and dilute to 100.0 ml with the same solvent, mix and filter.

Reference solution. A solution containing 0.06 per cent w/v of *amoxycillin trihydrate IPRS* and 0.012 per cent w/v of *lithium clavulanate IPRS* in *water*.

Chromatographic system
- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture 5 volumes of *methanol* and 95 volumes of *phosphate buffer pH 4.4*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to amoxycillin and clavulanic acid is not less than 3.5, the tailing factor is not more than 1.5, the column efficiency is not less than 550 theoretical plates for both component and the relative standard deviation is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$.

1 mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

Labelling. The label states the quantity of Amoxycillin Sodium contained in it, in terms of the equivalent amount of amoxycillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid.

Amoxycillin and Potassium Clavulanate Oral Suspension

Amoxicillin and Potassium Clavulanate Oral Suspension

Amoxycillin and Potassium Clavulanate Oral Suspension is a mixture of Amoxycillin Trihydrate and Potassium Clavulanate or Potassium Clavulanate Diluted with buffering agents and other excipients. It contains a suitable flavouring agent.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Amoxycillin and Potassium Clavulanate Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$ and not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of clavulanic acid, $C_8H_9NO_5$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amounts of amoxycillin, $C_{16}H_{19}N_3O_5S$ and clavulanic acid, $C_8H_9NO_5$.

Usual strength. Amoxycillin 200 mg and Clavulanic acid 28.5 mg per 5 ml.

Identification

In the Assay, the retention time of the two principal peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.8 to 6.6.

Water (2.3.43). Not more than 7.5 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is less than 40 mg per ml; not more than 8.5 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxicillin that is equal to or more than 40 mg per ml and is less than or equal to 50 mg per ml; not more than 11.0 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is more than 50 mg per ml and is less than or equal to 80 mg per ml; not more than 12.0 per cent where the label indicates that after reconstitution as directed, the suspension contains an amount of amoxycillin that is more than 80 mg per ml.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer a weighed quantity containing about 50 mg of amoxycillin to a 100-ml volumetric flask, dissolve in *water*, dilute to 100.0 ml with the same solvent and filter. Use the filtrate as the test solution within 1 hour.

Reference solution. A solution containing 0.05 per cent w/v of *amoxycillin trihydrate IPRS* and 0.0075 per cent w/v of *lithium clavulanate IPRS* in *water*.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture 5 volumes of *methanol* and 95 volumes of *phosphate buffer pH 4.4*,

- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The relative retention times are about 0.5 for clavulanic acid and 1.0 for amoxycillin. The resolution between the amoxycillin and clavulanic acid peaks is not less than 3.5. The test is not valid unless the column efficiency determined from each analyte peak is not less than 550 theoretical plates, the tailing factor for each analyte peak is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$, weight in volume.

1 mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states (1) the quantity of Amoxycillin Trihydrate contained in it, in terms of the equivalent amount of amoxycillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid; (2) the temperature of storage and the period during which the constituted suspension may be expected to be statisfactory use.

Amoxycillin and Potassium Clavulanate Tablets

Amoxicillin and Potassium Clavulanate Tablets

Amoxycillin and Potassium Clavulanate Tablets contain Amoxycillin Trihydrate and Potassium Clavulanate or Potassium Clavulanate Diluted. The tablets are coated.

Amoxycillin and Potassium Clavulanate Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amounts of amoxycillin, $C_{16}H_{19}N_3O_5S$ and clavulanic acid, $C_8H_9NO_5$.

Usual strength. Amoxycillin 500 mg and Clavulanic acid 125 mg.

Identification

In the Assay, the retention time of the two principal peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

Tests

Disintegration (2.5.1). 30 minutes, for tablets labelled for veterinary use only, simulated gastric juice, artificial being substituted for water in the test.

Dissolution (2.5.2). (*Tablets labelled for veterinary use only are exempt from this requirement*).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*,

Speed and time. 75 rpm and 30 minutes or 45 minutes where the Tablets are labelled as chewable.

Withdraw a suitable volume of the medium and filter. Carry out the method described under Assay.

Q. Not less than 85 per cent of the stated amount of $C_{16}H_{19}N_3O_5S$ and not less than 80 per cent of the stated amount of $C_8H_9NO_5$.

For tablets labelled as chewable. Not less than 80 per cent of the stated amount of the $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ is dissolved in 45 minutes.

Uniformity of content. Complies with the test stated under Tablets, determining the content of clavulanic acid in the tablets.

Use chromatographic procedure described under Assay using the following test solution.

Powder one tablet and transfer to a 100-ml flask. Dissolve in *water* and dilute to 100.0 ml with the same solvent and filter. Further dilute to obtain a solution containing 0.05 per cent w/v of amoxycillin. Use the solution within 1 hour.

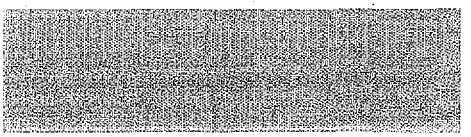
Calculate the content of $C_8H_9NO_5$ in the tablet.

Water (2.3.43). Not more than 7.5 per cent, where the labelled amount of amoxycillin in each tablet is 250 mg or less; not more than 10.0 per cent where the labelled amount of amoxycillin in each tablet is more than 250 mg but less than or equal to 500 mg; not more than 11.0 per cent where the labelled amount of amoxycillin in each tablet is more than 500 mg. Where the tablets are labelled as chewable, not more than 6.0 per cent where the labelled amount of amoxycillin in each tablet is 125 mg or less; not more than 8.0 per cent where the labelled amount of amoxycillin in each tablet is more than 125 mg. Where the tablets are labelled for veterinary use only, not more than 10.0 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powdered tablet containing about 50 mg of amoxycillin, dissolve in *water*, dilute to 100.0 ml with *water* and filter. Use the filtrate as the test solution within 1 hour.



Reference solution. A solution containing 0.05 per cent w/v of *amoxycillin trihydrate IPRS* and 0.013 per cent w/v of *lithium clavulanate IPRS* in *water*.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture 5 volumes of *methanol* and 95 volumes of *phosphate buffer pH 4.4*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The relative retention times are about 0.5 for clavulanic acid and 1.0 for amoxycillin. The resolution between the amoxycillin and clavulanic acid peaks is not less than 3.5. The test is not valid unless the column efficiency determined from each analyte peak is not less than 550 theoretical plates, the tailing factor for each analyte peak is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

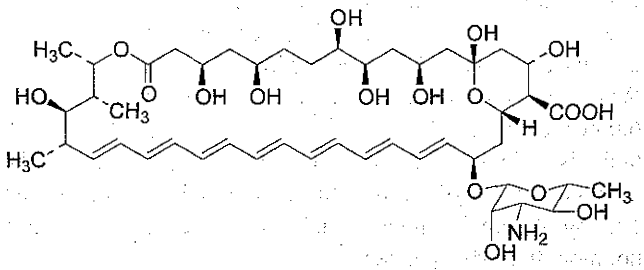
Calculate the content of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ in the tablets.

1 mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

Storage. Store protected from moisture.

Labelling. The label includes the word "chewable" in juxtaposition to the official name in the case of Chewable Tablets. The label also indicates that Chewable Tablets may be chewed before being swallowed or may be swallowed whole. Tablets intended for veterinary use only are so labelled.

Amphotericin B



$C_{47}H_{73}NO_{17}$ Mol. Wt. 924.1

Amphotericin B is a mixture consisting mainly of amphotericin B which is (3*R*,5*R*,8*R*,9*R*,11*S*,13*R*,15*S*,16*R*,17*S*,19*R*,34*S*,35*R*,36*R*,37*S*)-19-(3-amino-3,6-dideoxy-β-D-mannopyranosyloxy)-16-carboxy-3,5,8,9,11,13,15, 35-octahydroxy-34,36-dimethyl-13,17-epoxyoctatriaconta- 20,22,24,26,28,30,32-heptaen-37-olide and other antifungal polyenes produced by the growth of certain strains of *Streptomyces nodosus* or by any other means.

Amphotericin B has a potency of not less than 750 µg of $C_{47}H_{73}NO_{17}$ per mg, calculated on the dried basis.

Category. Antifungal.

Description. A yellow to orange powder. Even in the absence of light, it is gradually decomposed in a humid environment, degradation being faster at higher temperatures. In solutions, it is inactivated in the presence of light and at low pH values.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amphotericin B IPRS* or with the reference spectrum of amphotericin B.

B. Dissolve 25 mg in 5 ml of *dimethyl sulphoxide*, add sufficient *methanol* to produce 50 ml, and dilute 2 ml to 200 ml with *methanol*. When examined in the range 300 nm to 450 nm (2.4.7), the resulting solution shows absorption maxima at about 362 nm, 381 nm, and 405 nm. The ratio of the absorbance at the maximum at about 362 nm to the absorbance at the maximum at about 381 nm, 0.5 to 0.6; the ratio of the absorbance at the maximum at about 381 nm to the absorbance at the maximum at about 405 nm, about 0.9.

C. To 1 ml of a 0.05 per cent w/v solution in *dimethyl sulphoxide* add 5 ml of *phosphoric acid* to form a lower layer; a blue ring is immediately formed at the junction of the liquids. Mix; the mixture becomes intensely blue. Add 15 ml of *water* and mix; the solution becomes pale straw-coloured.

Tests

Tetraenes. Not more than 15.0 per cent (for parenteral use, not more than 10.0 per cent), determined by the following method. Weigh 50 mg, dissolve in 5 ml of *dimethyl sulphoxide*, dilute to 50.0 ml with *methanol* and dilute 4.0 ml of the resulting solution to 50.0 ml with *methanol* (solution 1). Prepare solution (2) in a similar manner using 50 mg of *amphotericin B IPRS*, weighed, instead of the substance under examination. For solution (3) dissolve 25 mg of *nystatin IPRS*, weighed, in 25 ml of *dimethyl sulphoxide*, dilute to 250.0 ml with *methanol* and dilute 4.0 ml to 50.0 ml with *methanol*. Using as the blank a 0.8 per cent v/v solution of *dimethyl sulphoxide* in *methanol*, measure the absorbances of solutions (1), (2) and (3) at the maxima at about 282 nm and about 304 nm (2.4.7).

Calculate the absorbance for the substance under examination, *amphotericin B IPRS* and *nystatin IPRS* at both wavelengths and calculate the content of tetraenes from the expression

$$\frac{25\,W_N[(A_{B282}\times A_{U304})-(A_{B304}\times A_{U282})}{[(A_{B282}\times A_{N304})-(A_{B304}\times A_{N282})]W_U}$$

where W_N is the weight, in mg, of nystatin RS, A_{B282} and A_{B304} are the absorbance of *amphotericin B IPRS* at about 282 nm and 304 nm, respectively, A_{N282} and A_{N304} are the absorbance of *nystatin IPRS* at about 282 nm and 304 nm respectively, A_{U282} and A_{U304} are the absorbance of the substance under examination at about 282 nm and 304 nm respectively and W_U is the weight in mg of the sample taken.

Sulphated ash (2.3.18). Not more than 3.0 per cent; for parenteral use, not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10). Express the result in µg per mg.

Amphotericin B intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.0 Endotoxin Unit per mg, using the supernatant liquid obtained after shaking 50 mg with 25 ml of *water BET* and centrifuging.

Storage. Store protected from light in a refrigerator (2° to 8°). Do not freeze.

Labelling. The label states (1) the number of Units per mg; (2) whether the material is intended for use in the manufacture of parenteral preparations.

Amphotericin B Injection

Amphotericin B Injection is a sterile freeze dried mixture of Amphotericin B and deoxycholate sodium with one or more buffering agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate Matter stated under Parenteral Preparations (Injections)

Storage. The constituted solution should be used immediately after preparation but, in any case within the period recommended by the manufacturer.

Amphotericin B Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amphotericin B, $C_{47}H_{73}NO_{17}$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strength. 50 mg per ml.

Identification

Dissolve a quantity of powder for injection containing 25 mg of Amphotericin B in 5 ml of *dimethyl sulphoxide*, add sufficient *methanol* to produce 50 ml, and dilute 2 ml to 200 ml with *methanol*. When examined in the range 300 nm to 450 nm (2.4.7), the resulting solution shows absorption maxima at about 362 nm, 381 nm, and 405 nm. The ratio of the absorbance at the maximum at about 362 nm to the absorbance at the maximum at about 381 nm, 0.5 to 0.6; the ratio of the absorbance at the maximum at about 381nm to the absorbance at the maximum at about 405 nm, about 0.9.

Tests

pH (2.4.24). 7.2 to 8.0 determined in a solution containing 10 mg per ml of Amphotericin B.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 0.1 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Bacterial Endotoxins (2.2.3). Not more than 5.0 Endotoxin unit per mg of amphotericin B. For products used or labelled for intrathecal injection, not more than 0.9 Endotoxin unit per mg.

Sterility (2.2.11). Complies with the test for sterility.

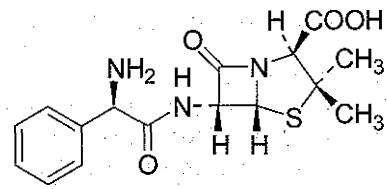
Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Mix the contents of 10 containers, dissolve in *dimethyl-sulphoxide*. Express the results in mg per vial, taking each 1000 µg found to be equivalent to 1 mg of amphotericin B.

Storage. Store in tightly closed containers between 2° to 8°, protected from light.

Labelling. Label it to state that it is intended for use by intravenous infusion to hospitalised patients only, and that the solution should be protected from light during administration.

Ampicillin



$C_{16}H_{19}N_3O_4S$ Mol. Wt. 349.4

Ampicillin is (6*R*)-6-(α-phenyl-D-glycylamino)penicillanic acid.

Ampicillin contains not less than 96.0 per cent and not more than 100.5 per cent of $C_{16}H_{19}N_3O_4S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin IPRS* or with the reference spectrum of ampicillin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. Dissolve 1.0 g in 10 ml of *1 M hydrochloric acid* and a further 1.0 g in a mixture of 3 ml of *dilute ammonia solution* and 7 ml of *water*. Both solutions when freshly prepared are not more opalescent than opalescence standard OS2 (2.4.1).

pH (2.4.24). 3.5 to 5.5, determined in a 0.25 per cent w/v solution.

Specific optical rotation (2.4.22). +280° to +305°, determined in a 0.25 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 27 mg of the substance under examination in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a). A 0.054 per cent w/v solution of *anhydrous ampicillin IPRS* in mobile phase A.

Reference solution (b). A 0.004 per cent w/v solution of *cefradine IPRS* in mobile phase A. To 5.0 ml of the solution, add 5.0 ml of reference solution (a).

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A.

Reference solution (d). To 0.2 g of the substance under examination, add 1.0 ml of *water*. Heat the solution at 60° for 1 hour. Dilute the solution to 50.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 0.5 ml of *dilute acetic acid*, 50 ml of *0.2 M potassium dihydrogen phosphate* and 50 ml of *acetonitrile*, dilute to 1000 ml with *water*,
  B. a mixture of 0.5 ml of *dilute acetic acid*, 50 ml of *0.2 M potassium dihydrogen phosphate* and 400 ml of *acetonitrile*, dilute to 1000 ml with *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
t_R	85	15
(t_R+30)	0	100
(t_R+45)	0	100
(t_R+60)	85	15

t_R is the retention time of ampicillin determined with reference solution (c).

Inject reference solution (b) with isocratic elution at the initial mobile phase composition to determine t_R.

The relative retention time with reference to ampicillin for ampicillin dimer is about 2.8.

Inject reference solution (b) and (d). The test is not valid unless in the chromatogram obtained with reference solution (b) the resolution between the peaks due to ampicillin and cefradine is not less than 3.0, if necessary adjust the ratio A:B of the mobile phase. The chromatogram obtained with reference solution (d) shows two peaks, due to ampicillin and ampicillin dimer.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

***N, N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2. 4.14).

Solvent mixture. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

Test solution. Transfer a weighed quantity containing about 0.1 g of ampicillin to a 100-ml volumetric flask, add about 80 ml of the solvent mixture, shake and mix with the aid of ultrasound if necessary to achieve complete dissolution and dilute to 100.0 ml with the solvent mixture. Use the solution promptly after preparation.

Reference solution (a). Weigh a suitable quantity of *ampicillin IPRS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a

solution having a known concentration of about 1 mg per ml. Use the solution promptly after preparation.

Reference solution (b). Dissolve *caffeine IPRS* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 90.9 volumes of *water*, 8 volumes of *acetonitrile*, 1 volume of *1 M monobasic potassium phosphate*, and 0.1 ml of *1 M acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The relative retention time with reference to caffeine for ampicillin is about 0.5.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_4S$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Ampicillin Capsules

Ampicillin Capsules contain Ampicillin or Ampicillin Trihydrate equivalent to not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

Usual strengths. 250 mg; 500 mg.

Identification

The contents of the capsules comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin IPRS* or with the reference spectrum of ampicillin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Use one capsule in the vessel for each test.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Transfer a measured portion of the filtrate, estimated to contain about 1 mg of ampicillin to a 50-ml volumetric flask, dilute with a 1 per cent v/v solution of *formaldehyde* in *0.3 M hydrochloric acid*. Heat the solution to 90° ± 5° in a constant temperature bath for 60 minutes. Measure the absorbance of the resulting solution at the maximum at about 352 nm (2.4.7). Calculate the content of $C_{16}H_{19}N_3O_4S$ in the medium from the absorbance obtained from a solution of known concentration of *ampicillin IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{19}N_3O_4S$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2. 4.14).

Solvent mixture. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing about 0.1 g of ampicillin, add about 80 ml of the solvent mixture and dissolve by shaking for 15 minutes and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use the solution promptly after preparation.

Reference solution (a). Weigh a suitable quantity of *ampicillin IPRS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use the solution promptly after preparation.

Reference solution (b). Dissolve *caffeine IPRS* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic micro particles (5 µm),
- mobile phase: a mixture of 90.9 volumes of *water*, 8 volumes of *acetonitrile*, 1 volume of *1 M monobasic potassium phosphate*, and 0.1 ml of *1 M acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_4S$ in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of ampicillin (when Ampicillin Trihydrate is used).

Ampicillin Oral Suspension

Ampicillin Oral Suspension is a mixture consisting of Ampicillin or Ampicillin Trihydrate with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before issue.

Ampicillin Oral Suspension contains Ampicillin Trihydrate equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

The constituted suspension, when stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use, contains not less than 80.0 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Usual strengths. Ampicillin 125 mg per 5 ml; Ampicillin 250 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 4.0 to 7.0.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2. 4.14).

Solvent mixture. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

Test solution. Transfer a weighed quantity containing about 0.1 g of ampicillin to a 100-ml volumetric flask and dilute to 100.0 ml with the solvent mixture and filter. Use the solution promptly after preparation.

Reference solution (a). Weigh a suitable quantity of *ampicillin IPRS,* dissolve in the solvent mixture by shaking and mixing with the aid of ultrasound if necessary, to obtain a solution having a known concentration of about 1 mg per ml. Use the solution promptly after preparation.

Reference solution (b). Dissolve *caffeine IPRS* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 90.9 volumes of *water,* 8 volumes of *acetonitrile,* 1 volume of *1 M monobasic potassium phosphate* and 0.1 ml of *1 M acetic acid,*
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{16}H_{19}N_3O_4S$, weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Labelling. The label states (1) the quantity of active ingredient in terms of the equivalent amount of ampicillin when the active ingredient is Ampicillin Trihydrate; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

Ampicillin Dispersible Tablets

Dispersible Ampicillin Tablets

Ampicillin Dispersible Tablets contain Ampicillin or Ampicillin Trihydrate in a suitable dispersible base.

Ampicillin Dispersible Tablets contain Ampicillin or Ampicillin Trihydrate equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

Usual strengths. The equivalent of 125 mg and 250 mg of ampicillin.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Uniformity of dispersion. Place 2 tablets in 100 ml of *water* and stir until completely dispersed. A smooth dispersion is produced, which passes through a sieve screen with a nominal mesh aperture of 710 µm.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2. 4.14).

Solvent mixture. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.1 g of ampicillin to a 100-ml volumetric flask, add about 80 ml of the solvent mixture, shake for 15 minutes and mix with the aid of ultrasound to achieve complete dissolution. Dilute to 100.0 ml with the solvent mixture and filter. Use the solution promptly after preparation.

Reference solution (a). Weigh a suitable quantity of *ampicillin IPRS,* dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use the solution promptly after preparation.

Reference solution (b). Dissolve *caffeine IPRS* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 90.9 volumes of *water,* 8 volumes of *acetonitrile,* 1 volume of *1 M monobasic potassium phosphate* and 0.1 ml of *1 M acetic acid,*
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The relative retention times with reference to ampicillin for caffeine is about 2.0.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

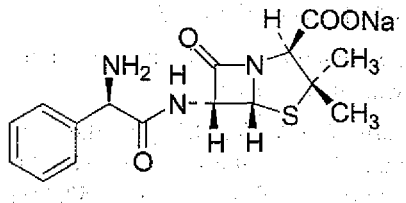
Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_4S$ in the tablets.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) the strength in terms of the equivalent amount of ampicillin (when Ampicillin Trihydrate is used); (2) that the tablets should be dispersed in water immediately before use.

Ampicillin Sodium



$C_{16}H_{18}N_3NaO_4S$ Mol. Wt. 371.4

Ampicillin Sodium is sodium (6*R*)-6-(α-phenyl-D-glycylamino)penicillinate

Ampicillin Sodium contains not less than 92.5 per cent and not more than 100.5 per cent of $C_{16}H_{18}N_3NaO_4S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white, crystalline powder; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin sodium IPRS* or with the reference spectrum of ampicillin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution is clear, when examined immediately after preparation (2.4.1), and the absorbance of the solution at about 430 nm (2.4.7) is not more than 0.15.

pH (2.4.24). 8.0 to 10.0, determined 10 minutes after dissolution in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). + 258° to + 287°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate.*

Related substances. Determine by liquid chromatography (2.4.14).

NNOTE —Prepare the solutions immediately before use.

Test solution. Dissolve 31 mg of the substance under examination in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a). A 0.054 per cent w/v solution of *anhydrous ampicillin IPRS* in mobile phase A.

Reference solution (b). A 0.004 per cent w/v solution of *cefradine IPRS* in mobile phase A. To 5.0 ml of the solution, add 5.0 ml of reference solution (a).

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A.

Reference solution (d). To 0.2 g of the substance under examination, add 1.0 ml of *water*. Heat the solution at 60° for 1 hour. Dilute 0.5 ml of the solution to 50.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 0.5 ml of *dilute acetic acid*, 50 ml of *0.2 M potassium dihydrogen phosphate* and 50 ml of *acetonitrile*, dilute to 1000 ml with *water*;
  B. a mixture of 0.5 ml of *dilute acetic acid*, 50 ml of *0.2 M potassium dihydrogen phosphate* and 400 ml of *acetonitrile*, dilute to 1000 ml with *water*;
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
t_R	85	15
(t_R+30)	0	100
(t_R+45)	0	100
(t_R+60)	85	15

t_R is the retention time of ampicillin determined with reference solution (c).

Inject reference solution (b) with isocratic elution at the initial mobile phase composition to determine t_R.

The relative retention time with reference to ampicillin for ampicillin dimer is about 2.8.

Inject reference solution (b) and (d). The test is not valid unless in the chromatogram obtained with reference solution (b) the resolution between the peaks due to ampicillin and cefradine is not less than 3.0, if necessary adjust the ratio A:B of the mobile phase. The chromatogram obtained with reference solution (d) shows two peaks, due to ampicillin and ampicillin dimer.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to ampicillin dimer is not more than 4.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4.5 per cent) and the area of any other secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

Dichloromethane. Not more than 0.2 percent w/w, determined by gas chromatography (2.4.13).

Test solution. Dissolve 250 mg of the substance under examination in 2.0 ml of *water* into a headspace vial.

Reference solution. A 0.026 per cent w/v solution of *dichloromethane* in *water*. Transfer 2.0 ml of the solution to a headspace vial.

Chromatographic system.

- a capillary column 30 m x 0.25 mm packed with 6.0 per cent polycyanopropylphenyl siloxane and 94.0 per cent of polydimethyl siloxane (1.4 µm) (Such as DB-624),
- temperature:
  column 50° for 10 minutes, 50° to 130° @ 12° per minute and hold at 130° for 5 minutes. Post run 220° for 5 minutes,
- inlet port at 180° and detector at 250°,
- flame ionization detector,
- split ratio: 25:1,
- flow rate: 0.5 ml per minute using nitrogen as carrier gas.

Headspace conditions

- incubation/equilibrium temperature: 80°,
- incubation /equilibrium time: 1200 seconds,
- syringe temperature/transfer line temperature: 90°,
- injection volume: 500 µl,

Inject the reference solution and the test solution.

The test is not valid unless the relative standard deviation for replicate injections is not more than 15.0 per cent

NOTE — Can be reduced to 8.0 per cent.

Calculate the content of dichloromethane.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2. 4.14).

Solvent mixture. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

Test solution. Transfer a weighed quantity containing about 0.1 g of ampicillin to a 100-ml volumetric flask and dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Use the solution promptly after preparation.

Reference solution (a). Weigh a suitable quantity of *ampicillin IPRS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use the solution promptly after preparation.

Reference solution (b). Dissolve *caffeine IPRS* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 90.9 volumes of *water*, 8 volumes of *acetonitrile*, 1 volume of *1 M monobasic potassium phosphate*, and 0.1 ml of *1 M acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{19}N_3NaO_4S$ by multiplying the content of $C_{16}H_{19}N_3O_4S$ by 1.063.

Ampicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial Endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg.

Ampicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Ampicillin Injection

Ampicillin Sodium Injection

Ampicillin Injection is a sterile material consisting of Ampicillin Sodium with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ampicillin Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

Category. Antibacterial

Usual strengths. The equivalent of 100 mg, 250 mg, 500 mg and 1 g of ampicillin.

Description. A white or almost white powder; hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injections) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin sodium IPRS* or with the reference spectrum of ampicillin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10 per cent w/v solution is clear, when examined immediately after preparation (2.4.1), and the absorbance of the solution at about 430 nm is not more than 0.15.

pH (2.4.24). 8.0 to 10.0, determined 10 minutes after dissolution in a 10 per cent w/v solution.

Specific optical rotation (2.4.22). + 258° to + 287°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE —Prepare the solutions immediately before use.

Test solution. Dissolve a quantity of the injection containing about 27 mg of the substance under examination in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a). A 0.054 per cent w/v solution of *anhydrous ampicillin IPRS* in mobile phase A.

Reference solution (b). A 0.004 per cent w/v solution of *cefradine IPRS* in mobile phase A. To 5.0 ml of the solution, add 5.0 ml of reference solution (a).

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A.

Reference solution (d). To a quantity of the injection containing about 0.2 g of ampicillin, add 1.0 ml of *water*. Heat the solution at 60° for 1 hour. Dilute 0.5 ml of the solution to 50.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 0.5 ml of *dilute acetic acid*, 50 ml of *0.2 M potassium dihydrogen phosphate* and 50 ml of *acetonitrile*, dilute to 1000 ml with *water*,
  B. a mixture of 0.5 ml of *dilute acetic acid*, 50 ml of *0.2 M potassium dihydrogen phosphate* and 400 ml of *acetonitrile*, dilute to 1000 ml with *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
t_R	85	15
(t_R+30)	0	100
(t_R+45)	0	100
(t_R+60)	85	15

t_R is the retention time of ampicillin determined with reference solution (c).

Inject reference solution (b) with isocratic elution at the initial mobile phase composition to determine t_R.

The relative retention time with reference to ampicillin for ampicillin dimer is about 2.8.

Inject reference solution (b) and (d). The test is not valid unless in the chromatogram obtained with reference solution (b) the resolution between the peaks due to ampicillin and cefradine is not less than 3.0, if necessary adjust the ratio A:B of the mobile phase. The chromatogram obtained with reference solution (d) shows two peaks, due to ampicillin and ampicillin dimer.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to ampicillin dimer is not more than 4.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4.5 per cent) and the area of any other secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

Dichloromethane. Not more than 0.2 percent w/w, determined by gas chromatography (2.4.13).

Test Solution. Dissolve 250 mg of the substance under examination in 2.0 ml of *water* into a headspace vial.

Reference solution. A 0.026 per cent w/v solution of dichloromethane in *water*. Transfer 2.0 ml of the solution to a headspace vial.

Chromatographic system.

- a capillary column 30 m x 0.25 mm packed with 6.0 per cent polycyanopropylphenyl siloxane and 94.0 per cent of polydimethyl siloxane (1.4 µm) (Such as DB-624),
- temperature:
  column 50° for 10 minutes, 50° to 130° @ 12° per minute and hold at 130° for 5 minutes. Post run 220° for 5 minutes,
- inlet port at 180° and detector at 250°,
- flame ionization detector,
- split ratio: 25:1,
- flow rate: 0.5 ml per minute using nitrogen as carrier gas.

Headspace conditions

- incubation/equilibrium temperature: 80°,
- incubation /equilibrium time: 1200 seconds,
- syringe temperature/transfer line temperature: 90°,
- injection volume: 500 µl,

Inject the reference solution and the test solution.

The test is not valid unless the relative standard deviation for replicate injections is not more than 15.0 per cent *(NOTE — can be reduced to 8.0 per cent)*.

Calculate the content of dichloromethane.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Bacterial Endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg of ampicillin.

Sterility (2.2.11). Complies with the test for sterility.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2. 4.14).

Solvent mixture. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

Test solution. Determine the weight of the contents of 10 containers. Transfer a weighed quantity of the mixed contents of the 10 containers containing 0.1 g of ampicillin to a 100-ml volumetric flask, add about 80 ml of the solvent mixture and dissolve by shaking and mixing if necessary, with the aid of ultrasound. Dilute to 100.0 ml with the solvent mixture and filter. Use the solution promptly after preparation.

Reference solution (a). Weigh a suitable quantity of *ampicillin IPRS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use the solution promptly after preparation.

Reference solution (b). Dissolve *caffeine IPRS* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 90.9 volumes of *water*, 8 volumes of *acetonitrile*, 1 volume of *1 M monobasic potassium phosphate* and 0.1 ml of *1 M acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

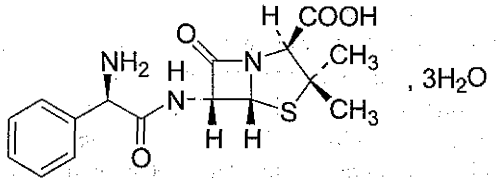
Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_4S$ in the injection.

Storage. Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°.

Labelling. The label states the quantity of Ampicillin Sodium contained in the sealed container in terms of the equivalent amount of anhydrous ampicillin.

Ampicillin Trihydrate



$C_{16}H_{19}N_3O_4S,3H_2O$ Mol. Wt. 403.5

Ampicillin Trihydrate is (6*R*)-6-(α-phenyl-D-glycylamino)penicillanic acid trihydrate.

Ampicillin Trihydrate contains not less than 96.0 per cent and not more than 100.5 per cent of $C_{16}H_{19}N_3O_4S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin trihydrate IPRS* or with the reference spectrum of ampicillin trihydrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. Dissolve 1.0 g in 10 ml of *1 M hydrochloric acid* and a further 1.0 g in a mixture of 3 ml of *dilute ammonia solution* and 7 ml of *water*. Both solutions when freshly prepared are not more opalescent than opalescence standard OS2 (2.4.1).

pH (2.4.24). 3.5 to 5.5, determined in a 0.25 per cent w/v solution.

Specific optical rotation (2.4.22). +280° to +305°, determined in a 0.25 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 31 mg of the substance under examination in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a). A 0.054 per cent w/v solution of *anhydrous ampicillin IPRS* in mobile phase A.

Reference solution (b). A 0.004 per cent w/v solution of *cefradine IPRS* in mobile phase A. To 5.0 ml of the solution, add 5.0 ml of reference solution (a).

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 0.5 ml of *dilute acetic acid*, 50 ml of *0.2 M potassium dihydrogen phosphate* and 50 ml of *acetonitrile*, diluted to 1000 ml with *water*,
  B. a mixture of 0.5 ml of *dilute acetic acid*, 50 ml of *0.2 M potassium dihydrogen phosphate* and 400 ml of *acetonitrile*, diluted to 1000 ml with *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
t_R	85	15
(t_R+30)	0	100
(t_R+45)	0	100
(t_R+60)	85	15

t_R is the retention time of ampicillin determined with reference solution (c).

Inject reference solution (b) with isocratic elution at the initial mobile phase composition to determine t_R.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to ampicillin and cefradine is not less than 3.0.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

NOTE — Test to be performed only if N,N Dimethylaniline is used in the synthesis.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). 12.0 per cent to 15.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2. 4.14).

Solvent mixture. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

Test solution. Transfer a weighed quantity containing about 0.1 g of ampicillin to a 100-ml volumetric flask and dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Use the solution promptly after preparation.

Reference solution (a). Weigh a suitable quantity of *ampicillin IPRS*, dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml. Use the solution promptly after preparation.

Reference solution (b). Dissolve *caffeine IPRS* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 90.9 volumes of *water*, 8 volumes of *acetonitrile*, 1 volume of *1 M monobasic potassium phosphate*, and 0.1 ml of *1 M acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention times are about 0.5 for ampicillin and 1.0 for caffeine.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{19}N_3O_4S$.

Storage. Store at a temperature not exceeding 30°.

Alpha Amylase

Diastase

Alpha Amylase is an amylolytic enzyme or a mixture of enzymes obtained from fungi such as *Aspergillus oryzae* or from a non-pathogenic variant of bacteria such as *Bacillus subtilis* and with the specific activity for converting starch into dextrin and maltose. It may contain suitable harmless diluents such as Lactose or Dibasic Calcium Phosphate.

Alpha Amylase has amylase activity of not less than 800 Units which represents the number of grams of dry, soluble maize or corn starch digested by 1.0 g of Alpha Amylase under the conditions of the Assay.

Category. Digestive enzyme

Description. A cream to light brown-coloured powder; hygroscopic.

Tests

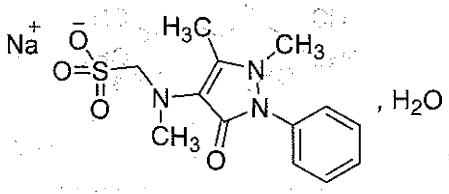
Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

Assay. Weigh a quantity containing 100 Units of amylase activity and triturate with 200 ml of *buffer solution pH 6.0* (*for bacterial amylase*) or of *acetate buffer pH 5.0* (*for fungal amylase*) and add sufficient *buffer solution pH 6.0* or *acetate buffer pH 5.0*, as appropriate, to produce 1000.0 ml. Dilute 10.0 ml to 100.0 ml with *buffer solution pH 6.0* or *acetate buffer pH 5.0*, as appropriate, to give the test solution; filter if necessary (1 ml of the test solution should be capable of digesting about 10 mg of dry soluble maize or corn starch). Into each of six stoppered test-tubes add 5.0 ml of *starch substrate* without touching the sides of the test-tube. Place the test-tubes in a water-bath maintained at 40° ± 0.1°. When the temperature of the solution in the tubes has reached 40°, add 0.35 ml, 0.4 ml, 0.45 ml, 0.5 ml, 0.55 ml and 0.6 ml of the test solution to each of the test-tubes marked 1 to 6 respectively and record the time of addition. Mix thoroughly and replace the tubes in the water-bath. After exactly 60 minutes remove the tubes and cool rapidly in cold *water*. Add to each tube 0.05 ml of *0.02 M iodine* and mix well. Note the tube containing the lowest volume of test solution that does not show a bluish or violet tinge (if there is doubt, warm the solution slightly, when the colour distinction is prominent). From this volume calculate the number of grams of dry soluble maize or corn starch digested by 1.0 g of the substance under examination. This represents the number of Units of amylase activity per g.

Storage. Store protected from light and moisture.

Analgin

Metamizole Sodium Monohydrate; Dipyrone



$C_{13}H_{16}N_3NaO_4S,H_2O$ Mol. Wt. 351.4

Analgin is Sodium [*N*-(2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1*H*-pyrazol-4yl)-*N*-methylamino]methanesulphonate monohydrate.

Analgin contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{13}H_{16}N_3NaO_4S$, calculated on the dried basis.

Category. Analgesic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metamizole sodium IPRS* or with the reference spectrum of metimazole sodium.

B. Dissolve 50 mg in 1 ml of *hydrogen peroxide solution(100 vol)*. A blue color is produced which fades rapidly and turns to intense red in a few minutes.

C. Dissolve 100 mg in 1.5 ml of *water* in test tube and add some glass beads. Add 1.5 ml of dilute *hydrochloric acid* and place a filter paper wetted with a solution prepared by 20 mg of *potassium iodate* in 2 ml of *starch solution* at the open end of the test tube. Heat gently, the evolving vapour of *sulphur dioxide* colours the filter paper blue. After heating gently for 1 min, take a glass rod with a drop of a solution prepared by 10 g of *chromotropic acid*, *sodium salt* in 1000 ml of *sulphuric acid* and place in the opening of the tube. Within 10 minutes, a blue-violet colour develops in the drop of the reagent.

D. 0.5 ml of solution A (See tests) gives reaction (A) of sodium (2.3.1).

Tests

Solution A. A 5.0 per cent w/v solution in *carbon dioxide free water*.

Appearance of solution. Solution A is clear (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

Acidity or alkalinity. To 5 ml of solution A, add 0.1 ml of *phenolphthalein solution*. The solution is colourless. Not more than 0.1 ml of *0.02 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of substance under examination in *methanol* and dilute to 10.0 ml with *methanol*.

Reference solution (a). Dissolve 5 mg of *metamizole impurity A IPRS* in *methanol* and dilute to 10.0 ml with *methanol*.

Reference solution (b). Dissolve 5 mg of *metamizole impurity E IPRS* in *methanol* and dilute to 10.0 ml with *methanol*.

Reference solution (c). In order to prepare metamizole impurity C in situ, dissolve 40 mg of substance under examination in *methanol*, dilute to 20.0 ml with *methanol* and boil under reflux for 10 minutes. Allow to cool to room temperature and dilute to 20.0 ml with *methanol*.

Reference solution (d). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol*.

Reference solution (e). Mix 0.4 ml each of reference solution (a) and reference solution (b) and dilute to 20.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (1.8µm),
- mobile phase: a mixture of 28 volumes of *methanol* and 72 volumes of buffer solution prepared by mixing 1000 volumes of 0.6 per cent w/v solution of *sodium dihydrogen phosphate* and 1 volume of *triethylamine*, adjusted to pH 7.0 with strong *sodium hydroxide solution*.
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5µl.

Name	Relative retention time	Correction factor
Metamizole impurity A[1]	0.6	—
Metamizole impurity E[2]	0.7	1.5
Metamizole (Retention time: about 2 minutes)	1.0	—
Metamizole impurity C[3]	2.9	—

[1]4-(formylamino)-1,5-dimethyl-2-phenyl-2,3-dihydro-1*H*-pyrazol-3-one,

[2][(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]methanesulfonic acid (4-*N*-desmethylmetamizole),

[3]1,5-dimethyl-4-(methylamino)-2-phenyl-2,3-dihydro-1*H*-pyrazol-3-one.

Inject reference solution (e). The test is not valid unless the peak to valley ratio is not less than 3.0

Inject reference solution (c) and (e). Use chromatogram obtained with reference solution (e) to identify the peak due to impurities A and E, use the chromatogram obtained with reference solution (c) to identify the peak due impurity C.

Inject reference solution (d) and the test solution. Run the chromatogram 4.5 times the retention time of the principal peak.In the chromatogram obtained with the test solution, the area of any peak corresponding to metimazole impurity C is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of any peak corresponding to metimazole impurity E is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (d) (0.15 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (d) (0.05 per cent). The sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (d) (0.5 per cent). Ignore any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (d) (0.03 per cent).

Sulphates (2.3.17). Not more than 0.1 per cent, dissolving 0.15 g in *water* and dilute to 15.0 ml with *water*.

Heavy metals (2.3.13). Dissolve 2 g of substance under examination in 20 ml of *water*.12 ml of the solution complies with the limit test for heavy metals, Method D (20 ppm), using 10 ml of lead standard solution (2 ppm Pb).

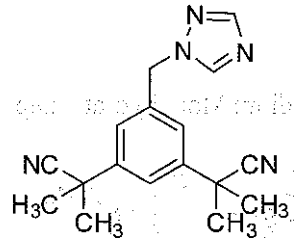
Loss on drying (2.4.19). 4.9 per cent to 5.3 per cent, determined on 1 g by drying in an oven at 105°.

Assay. Dissolve 200 mg of substance under examination in 10 ml of *0.01 M hydrochloric acid* previously cooled in ice *water* and titrate immediately, dropwise with *0.05 M iodine*. Before each addition of *0.05 M iodine* dissolve the precipitate by swirling. At the end of the titration, add 2 ml of *starch solution* and titrate until the blue colour of the solution persists for at least 2 min. The temperature of the solution during the titration must not exceed 10°.

1 ml of *0.05 M iodine* is equivalent to 0.01667 g of $C_{13}H_{16}N_3NaO_4S$.

Storage. Store protected from light.

Anastrozole



$C_{17}H_{19}N_5$ Mol. Wt. 293.4

Anastrozole is α,α,α′,α′-tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-benzene diacetonitrile

Anastrozole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{19}N_5$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A white to off white, crystalline powder.

CAUTION — Anastrozole is cytotoxic; extra care required to prevent inhaling particles and exposing the skin to it.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *anastrozole IPRS* or with the reference spectrum of anastrozole.

Tests

Melting range (2.4.21). 81° to 84°.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group (5 µm),
- mobile phase: a mixture of 65 volumes of *water*, 35 volumes of *acetonitrile* and 0.5 volume of *orthophosphoric acid*, adjusted to pH 3.0 with *1 M sodium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the peak in the chromatogram obtained with the reference solution (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the solvent mixture. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *anastrozole IPRS* in the solvent mixture.

Use the chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_5$.

Storage. Store protected from light and moisture.

Anastrozole Tablets

Anastrozole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anastrozole, $C_{17}H_{19}N_5$.

Usual strength. 1 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium to obtain a solution containing 0.0001 per cent w/v of Anastrozole.

Reference solution (a). A 0.01 per cent w/v solution of *anastrazole IPRS* in *acetonitrile*. Dilute 1.0 ml of the solution to 100.0 ml with the dissolution medium.

Reference solution (b). A solution containing 0.01 per cent w/v of *anastrozole IPRS* and 0.003 per cent w/v of *methyl paraben IPRS* in *acetonitrile*. Dilute 1.0 ml of the solution to 100.0 ml with the dissolution medium.

Chromatographic system

- a stainless steel column 10 cm x 3.2 mm, packed with octadecylsilane bonded to multi-alkyl silica (5 µm) (Such as Hichrom RPB C18),
- mobile phase: a mixture of 70 volumes of *water*, 30 volumes of *acetonitrile* and 0.1 volume of *trifluoro acetic acid*,

– flow rate: 0.75 ml per minute,
– spectrophotometer set at 215 nm,
– injection volume: 100 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to methylparaben and anastrozole is not less than 4.0,

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{19}N_5$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{19}N_5$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Disperse 1 tablet in 10.0 ml of the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *anastrozole IPRS* in the solvent mixture.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 10 mg of Anastrozole, shake with 70 ml of the solvent mixture, dilute to 100.0 ml with the solvent mixture and filter.

Reference solution. A 0.01 per cent w/v solution of *anastrozole IPRS* in the solvent mixture.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with phenyl group (5 µm),
– mobile phase: a mixture of 65 volumes of *water*, 35 volumes of *acetonitrile* and 0.5 volume of *orthophosphoric acid*, adjusted to pH 3.0 with *1 M sodium hydroxide*,
– flow rate: 1.5 ml per minute,
– spectrophotometer set at 220 nm,
– injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000 and tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_5$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Anticoagulant Citrate Dextrose Solution

ACD Solution

Anticoagulant Citrate Dextrose Solution is a sterile solution of Sodium Citrate, Citric Acid and Dextrose in Water for Injections.

Anticoagulant Citrate Dextrose Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of sodium citrate, $C_6H_5Na_3O_7,2H_2O$ and dextrose, $C_6H_{12}O_6,H_2O$, not less than 90.0 per cent and not more than110.0 per cent of the stated amount of citric acid monohydrate, $C_6H_8O_7,H_2O$ (or anhydrous citric acid, $C_6H_8O_7$),. It contains no antimicrobial agent.

Category. Anticoagulant for storage of whole blood.

Usual strengths.

	Solution A	Solution B
Sodium Citrate	2.20 g	1.32 g
Citric Acid (Anhydrous)	0.73 g	0.44 g
or Citric Acid (Monohydrate)	0.80 g	0.48 g
Dextrose (Monohydrate)	2.45 g	1.47 g
Water for Injection	100 ml	100 ml

NOTE — 15 ml of solution A or 25 ml of solution B are to be used for 100 ml of whole blood.

Description. A clear, colourless or faintly straw-coloured liquid.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. It gives the reactions of sodium salts (2.3.1).

C. To 2 ml (for Solution A) add 3 ml of *water* or to 4 ml (for Solution B) add 1 ml of *water*. The resulting solution gives reaction (A) of citrates (2.3.1).

Tests

pH (2.4.24). 4.5 to 5.5.

Sterility (2.2.11). Complies with the test for sterility.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial Endotoxins (2.2.3). Not more than 5.56 Endotoxin Units per ml.

Assay. *For sodium citrate* — Pipette 50.0 ml into a beaker and titrate with *1.3 M hydrochloric acid* to a pH of 1.98 ± 0.02, determining the end-point potentiometrically (2.4.25). Carry out a blank titration with 50 ml of *water*.

1 ml of *1.3 M hydrochloric acid* is equivalent to 0.1274 g of $C_6H_5Na_3O_7,2H_2O$.

For free citric acid — Pipette 20.0 ml into a conical flask and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.006404 g of $C_6H_8O_7$ or 0.007005 g of $C_6H_8O_7,H_2O$.

For dextrose — Determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation multiplied by 1.0425, represents the weight of $C_6H_{12}O_6,H_2O$ in 100 ml of the solution.

Storage. Store protected from light in a single dose, tamper-evident container of colourless, transparent glass or of a suitable plastic material.

Labelling. The label states (1) whether the contents are Solution A or Solution B; (2) volume of the solution required per 100 ml of whole blood or the volume of the solution required per volume of whole blood to be collected; (3) where applicable, the maximum amount of blood to be collected in the container.

Anticoagulant Citrate Phosphate Dextrose Solution

CPD Solution

Anticoagulant Citrate Phosphate Dextrose Solution is a sterile solution of Sodium Citrate, Citric Acid, Sodium Dihydrogen Phosphate Dihydrate and Dextrose in Water for Injection.

Anticoagulant Citrate Phosphate Dextrose Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of sodium citrate, $C_6H_5Na_3O_7,2H_2O$, sodium dihydrogen phosphate dihydrate, $NaH_2PO_4,2H_2O$ and dextrose, $C_6H_{12}O_6,H_2O$, not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of citric acid monohydrate, $C_6H_8O_7,H_2O$ (or anhydrous citric acid, $C_6H_8O_7$). It contains no antimicrobial agent.

Category. Anticoagulant for storage of whole blood.

Usual strengths.

Sodium Citrate	2.630 g
Citric Acid (Monohydrate)	0.327 g
Dextrose (Monohydrate)	2.550 g
Sodium Dihydrogen Phosphate (Dihydrate)	0.251 g
Water for Injection to	100 ml

NOTE — 14 ml are to be used for 100 ml of whole blood.

Description. A clear, colourless or faintly straw-coloured liquid.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. It gives the reactions of sodium salts and reaction (B) of phosphates (2.3.1).

C. To 2 ml add 3 ml of *water*. The resulting solution gives reaction (A) of citrates (2.3.1).

Tests

pH (2.4.24). 5.0 to 6.0.

Sterility (2.2.11). Complies with the test for sterility.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial Endotoxins (2.2.3). Not more than 5.56 Endotoxin Units per ml.

Assay. *For sodium citrate* — Dilute 25.0 ml to 100.0 ml with *water* and mix. Dilute 5.0 ml of the resulting solution to 100.0 ml with *water* and mix. Transfer 1.0 ml of the solution to a test-tube, add 1.3 ml of *pyridine*, swirl to mix, add 5.7 ml of *acetic anhydride*, mix and immediately place in a water-bath at 31° ± 0.5°. Allow the colour to develop for 35 minutes and measure the absorbance of the resulting solution at about 425 nm (2.4.7) using as the blank solution 1 ml of *water* treated in the same manner. Prepare a calibration curve by measuring the absorbance of solutions prepared by treating in the same manner 1 ml quantities of suitable dilutions of a solution in *water* containing 2.5 mg per ml of $C_6H_8O_7$, prepared by using *anhydrous citric acid*, previously dried for 3 hours at 90°. Calculate the total citrate content, as $C_6H_8O_7$, in mg per ml of the solution under examination from the expression 0.2 C, where C is the concentration in µg per ml of $C_6H_8O_7$, read from the curve.

Calculate the quantity, in mg, of $C_6H_5Na_3O_7,2H_2O$ in 1 ml of the solution under examination from the expression 1.53 $(A - B)$, where A is the concentration in mg per ml of total citrate as $C_6H_8O_7$ and B is the concentration in mg per ml of free citric acid in the solution.

For free citric acid — Pipette 20.0 ml into a conical flask and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

From the volume of *0.1 M sodium hydroxide* required subtract a volume, in ml, equal to 1.28 times the number of mg of $NaH_2PO_4,2H_2O$ present, as determined in the Assay for sodium acid phosphate.

1 ml of the remainder is equivalent to 0.007005 g of $C_6H_8O_7,H_2O$.

For sodium dihydrogen phosphate dihydrate — Dilute 5.0 ml to 100.0 ml with *water*. Transfer 5.0 ml to a 25-ml graduated flask and add 10.0 ml of a 2.8 per cent w/v solution of *sulphuric acid* followed by 2.0 ml of a 2.5 per cent w/v solution of *ammonium molybdate*, mixing after each addition. Add 1.0 ml of *aminohydroxynaphthalenesulphonic acid solution* and

sufficient *water* to produce 25.0 ml, mix and keep aside at 25° for 10 minutes. Measure the absorbance (A_1) of the resulting solution at the maximum at about 660 nm (2.4.7) using as the blank 5 ml of *water* treated in the same manner. Calculate the content of $NaH_2PO_4,2H_2O$ in each ml of the solution under examination from the absorbance (A_2) obtained by simultaneously carrying out the operation using 5.0 ml of a solution of *potassium dihydrogen phosphate* containing 0.11 mg of KH_2PO_4 per ml (C) and from the expression

$$22.92\ C\ (A_1/A_2).$$

For dextrose — Weigh a clean, medium- porosity sintered-glass crucible containing a few glass beads. To 50 ml of *potassium cupri-tartrate solution* add the glass beads from the weighed crucible, 45 ml of *water* and 5.0 ml of the solution under examination. Heat the solution at such a rate that it begins to boil in 3.5 to 4 minutes, boil the solution for exactly 2 minutes and filter immediately through the weighed crucible, taking care to transfer all the glass beads to the crucible, along with the precipitate. Wash the precipitate with hot *water* and then with 10 ml of *ethanol (95 per cent)* and dry it to constant weight at 110°. Carry out a blank determination.

1 mg of the precipitate is equivalent to 0.000496 g of $C_6H_{12}O_6,H_2O$.

Storage. Store in a single dose, tamper-evident container of colourless, transparent glass or of a suitable plastic material, protected from light.

Labelling. The label states (1) the composition and volume of the solution; (2) volume of the solution required per 100 ml of whole blood or the volume of the solution required per volume of whole blood to be collected; (3) where applicable, the maximum amount of blood to be collected in the container.

Anticoagulant Citrate Phosphate Dextrose Adenine Solution

CPDA Solution

Anticoagulant Citrate Phosphate Dextrose Adenine Solution is a sterile solution of Citric Acid, Sodium Citrate, Sodium Dihydrogen Phosphate Dihydrate, Dextrose and Adenine in Water for Injections.

Anticoagulant Citrate Phosphate Dextrose Adenine Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of total Sodium, Na, Sodium Dihydrogen Phosphate Dihydrate, $NaH_2PO_4,2H_2O$ and Dextrose Monohydrate, $C_6H_{12}O_6,H_2O$, not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of total Citrate, $C_6H_5O_7$ and Adenine, $C_5H_5N_5$. It contains no antimicrobial agent.

Category. Anticoagulant for storage of whole blood.

Usual strengths.

Citric Acid (Anhydrous)	0.2990 g
Sodium Citrate (Dihydrate)	2.6300 g
Sodium Dihydrogen Phosphate (Dihydrate)	0.2510 g
Adenine	0.0275 g
Dextrose (Monohydrate)	3.1900 g
Water for Injection to	100 ml

NOTE — 14 ml is to be used for 100 ml of whole blood.

Description. A clear, colourless or faintly straw-coloured liquid.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. It gives the reaction (B) of phosphates and the reactions of sodium salts (2.3.1).

C. To 2 ml add 3 ml of *water*. The resulting solution gives reaction (A) of citrates (2.3.1).

D. In the test for adenine in the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (c).

Tests

pH (2.4.24). 5.0 to 6.0.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 5.56 Endotoxin Units per ml.

Assay. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.3), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For total citrate — Dilute 5.0 ml of the solution under examination to 1000.0 ml with *water* and mix. Transfer 1.0 ml of the solution to a test-tube, add 1.3 ml of *pyridine*, swirl to mix, add 5.7 ml of *acetic anhydride*, mix and immediately place in a water-bath at 31° ± 1°. Allow the colour to develop for 33 ± 1 minutes and measure the absorbance of the resulting solution at about 425 nm (2.4.7), using as the blank 1 ml of *water* treated in the same manner. Prepare a calibration curve by measuring the absorbance of the solutions prepared by treating in the same manner 1 ml quantities of suitable dilutions of a solution in *water* containing 1.0 mg per ml of $C_6H_8O_7$,

prepared by using *anhydrous citric acid*, previously dried for 3 hours at 90°. Calculate the total citrate content, as $C_6H_8O_7$, in mg per ml of the solution under examination from the expression 0.2 C, where C is the concentration in µg per ml of $C_6H_8O_7$, read from the curve.

For sodium dihydrogen phosphate dihydrate — Dilute 5.0 ml to 100.0 ml with *water*. Transfer 5.0 ml of the solution to a 25-ml volumetric flask and add 10.0 ml of a 2.8 per cent w/v solution of *sulphuric acid* followed by 2.0 ml of a 2.5 per cent w/v solution of *ammonium molybdate*, mixing after each addition. Add 1.0 ml of *aminohydroxynaphthalenesulphonic acid solution* and sufficient *water* to produce 25.0 ml. Mix and keep aside at 25° for 10 minutes. Measure the absorbance (A_1) of the resulting solution at about 660 nm (2.4.7), using as the blank 5 ml of *water* treated in the same manner. Calculate the content of $NaH_2PO_4,2H_2O$ in each ml of the solution under examination from the absorbance (A_2) obtained by simultaneously carrying out the operation using 5.0 ml of a solution of *potassium dihydrogen phosphate* containing 0.11 mg of KH_2PO_4 per ml (C) using the expression

$$22.92\ C\ (A_1/A_2).$$

For adenine — Determine by liquid chromatography (2.4.14).

Test solution. Substance under examination.

Reference solution (a), (b) and (c) are prepared by dissolving weighed quantities of *adenine IPRS* in *dilute hydrochloric acid* in three separate volumetric flasks, diluting with the same solvent to volume and mixing to obtain reference solutions having known concentrations of about 0.25 mg, 0.275 mg and 0.30 mg of adenine per ml respectively.

Reference solution (d). A solution containing 0.0275 per cent w/v each of *adenine IPRS* and *purine* in *dilute hydrochloric acid.*

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with irregular or spherical, totally porous silica gel (10 µm) having a chemically bonded strongly acidic cation-exchange coating,
- mobile phase: dissolve 3.45 g of *ammonium dihydrogen phosphate* in 950 ml of *water* in a 1000-ml volumetric flask, add 10 ml of *glacial acetic acid*, dilute to volume with *water* and mix,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (d) at least four times and record the chromatograms. The test is not valid unless the relative standard deviation of the peak response of adenine is not more than 2.5 per cent, the relative standard deviation of the retention time of adenine is not more than 2.0 per cent and the resolution factor of adenine and purine is not less than 3.0.

Inject separately the test solution and reference solution (a), (b) and (c). Record the chromatograms and measure the responses for the major peaks. Plot the responses against the concentrations in mg of adenine per ml of reference solution (a), (b) and (c).

Calculate the quantity, in mg, of $C_5H_5N_5$ in each ml of the solution under examination as the value read directly from the standard curve corresponding to the response obtained with the test solution.

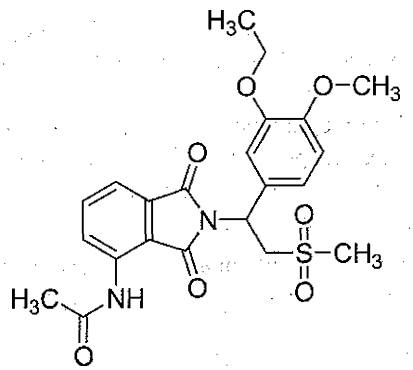
For dextrose — Weigh a clean, medium porosity sintered-glass crucible containing a few glass beads. To 50 ml of *potassium cupri-tartrate solution* add the glass beads from the weighed crucible, 45 ml of *water* and 5.0 ml of the solution under examination. Heat the solution at such a rate that it begins to boil in 3.5 to 4 minutes, boil the solution for exactly 2 minutes and filter immediately through the weighed crucible, taking care to transfer all the glass beads with the precipitate to the crucible. Wash the precipitate with hot *water* and then with 10 ml of *ethanol (95 per cent)* and dry it to constant weight at 110°. Carry out a blank determination.

1 mg of the precipitate is equivalent to 0.000496 g of $C_6H_{12}O_6.H_2O$.

Storage. Store protected from light, in single dose, tamper-proof containers made of a suitable plastic material in a cool place.

Labelling. The label states (1) the composition and volume of the solution; (2) volume of the solution required per 100 ml of whole blood or the volume of the solution required per volume of whole blood to be collected; (3) where applicable, the maximum amount of blood to be collected in the container.

Apremilast



$C_{22}H_{24}N_2O_7S$ Mol. Wt. 460.5

Apremilast is N-(2-(1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl)-1,3-dioxoisoindolin-4-yl) acetamide.

Apremilast contains not less than 98.0 per cent and not more than 102. 0 per cent of $C_{22}H_{24}N_2O_7S$, calculated on the dried basis.

Category. Anti-inflammatory.

Description. A white to pale yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *apremilast IPRS* or with the reference spectrum of apremilast.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of *acetonitrile*.

Reference solution (a). A solution containing 0.02 per cent w/v of *apremilast IPRS* and 0.0001 per cent w/v of *apremilast impurity B ((S)-4amino-2(1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl)isoindoline-1,3-dione) IPRS* in *acetonitrile*.

Reference solution (b). A 0.003 per cent w/v solution of *apremilast IPRS* in *acetonitrile*. Dilute 1.0 ml of the solution to 50.0 ml with *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group bonded to porous silica (5 µm) (Such as Zorbax SB phenyl),
- mobile phase: A. a 0.05 per cent v/v solution of *trifluoroacetic acid*,
  B. a mixture of 70 volumes of *acetonitrile*, 30 volumes of *methanol* and 0.05 volume of *trifluoroacetic acid*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0.01	95	5
30	10	90
45	10	90
45.1	95	5
50	95	5

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to apremilast impurity B and apremilast is not less than 1.5 in the chromatogram obtained with reference solution (a), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with test solution, the area of any peak corresponding to apremilast impurity B, multiplied by correction factor 1.54, is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any other secondary peak is not more than 0.33 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 1.67 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.17 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chiral purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 10 ml of *methanol* and dilute to 25.0 ml with *methanol*.

Reference solution. A solution containing 0.2 per cent w/v of *apremilast IPRS* and 0.001 per cent w/v of *apremilast R-isomer IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with cellulose tris (3,5-dimethylphenylcarbamate) bonded to porous silica (10 µm) (Such as Chiralcel OD),
- column temperature: 40°,
- mobile phase: a mixture of 60 volumes of *hexane*, 20 volumes of *isopropanol* and 20 volumes of *ethanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to apremilast *R*-isomer and *S*- isomer is not less than 1.5.

Inject the test solution. The area of any peak corresponding to *R*-isomer is not more than 0.5 per cent, calculated by area normalization.

Acetic acid content. Not more than 0.5 per cent.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution. A 0.025 per cent w/v solution of *sodium acetate trihydrate* in the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with polyvinyl alcohol with quarternary ammonium groups (5 µm) (Such as Metrosep A Supp-5),
- mobile phase: a solution of *1.5 mM sodium carbonate* containing *0.3 mM sodium bicarbonate*,
- flow rate: 0.7 ml per minute,
- conductivity detector,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of CH_3COOH by multiplying the content of $C_2H_9NaO_5$ with 0.44.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 100.0 ml of *acetonitrile*.

Reference solution. A 0.01 per cent w/v solution of *apremilast IPRS* in *acetonitrile*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group bonded to porous silica (5 µm) (Such as Zorbax SB phenyl),
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.05 volume of *trifluoroacetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}N_2O_7S$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Apremilast Tablets

Apremilast Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of apremilast, $C_{22}H_{24}N_2O_7S$.

Usual strengths. 10 mg; 20 mg and 30 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of phosphate buffer prepared by dissolving 3.4 g of *monobasic sodium phosphate* in 100 ml of *water*, adjusted to pH 6.8 with *1 M sodium hydroxide*, add 5 g of *sodium lauryl sulphate*, mix.

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 95 volumes of *acetonitrile* and 5 volumes of *water*.

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve a quantity of *apremilast IPRS* in the solvent mixture, and dilute with the dissolution medium to obtain a solution having similar concentration to the test solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 1 volume of *trifluoroacetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 25 µl.

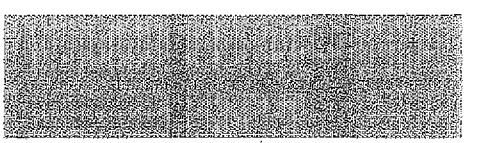
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}N_2O_7S$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{22}H_{24}N_2O_7S$.

Related substances. Determine by liquid chromatography (2.4.14).



Solvent mixture. 95 volumes of *acetonitrile* and 5 volumes of *water*.

Test solution. Disperse a quantity of the powdered tablets containing 20 mg of Apremilast in 5 ml of *water* and add 75 ml of the solvent mixture, sonicate for 20 minutes and dilute to 100.0 ml with the solvent mixture, centrifuge and use the clear supernatant liquid.

Reference solution (a). A 0.01 per cent w/v solution of *apremilast IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *apremilast impurity B ((S)-4 amino -2(1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl)isoindoline-1,3-dione) IPRS* and 0.02 per cent w/v of *apremilast IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group bonded to porous silica (5 µm) (Such as Zorbax SB Phenyl),
- sample temperature: 10°,
- mobile phase: A. a 0.05 per cent v/v solution of *trifluoroacetic acid*,
  B. a mixture of 70 volumes of *acetonitrile*, 30 volumes of *methanol* and 0.025 volume of *trifluoroacetic acid*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
30	10	90
45	10	90
45.1	95	5
50	95	5

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to apremilast impurity B and apremilast is not less than 1.5 in the chromatogram obtained with reference solution (b), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to apremilast impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in 5 ml of *water*, add 75 ml of *acetonitrile*, sonicate for 30 minutes and dilute to 100.0 ml with *acetonitrile*. Centrifuge and dilute a suitable volume of the supernatant liquid with 95 per cent v/v of *acetonitrile* to obtain a solution having similar concentration to the reference solution.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}N_2O_7S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 5 mg of Apremilast in 5 ml of *water* and add 75 ml of *acetonitrile*, sonicate for 10 minutes and dilute to 100.0 ml with *acetonitrile*, centrifuge and use the clear supernatant liquid.

Reference solution. A 0.005 per cent w/v solution of *apremilast IPRS* in 95 per cent v/v of *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group bonded to porous silica (5 µm) (Such as Zorbax SB Phenyl),
- mobile phase: a mixture of 10 volumes of *water*, 10 volumes of *acetonitrile* and 0.01 volume of *trifluoroacetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

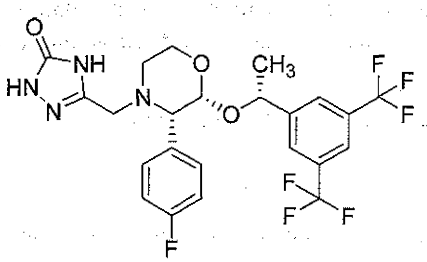
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}N_2O_7S$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Aprepitant



$C_{23}H_{21}F_7N_4O_3$ Mol. Wt. 534.4

Aprepitant is 3*H*-1,2,4-triazol-3-one, 5-[[(2*R*,3S)-2-[(1*R*)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro.

Aprepitant contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{23}H_{21}F_7N_4O_3$, calculated on the anhydrous and solvent-free basis.

Category. Antiemetic.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aprepitant IPRS* or with the reference spectrum of aprepitant.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). + 66.0° to + 71.0°, determined in a 1.0 per cent w/v solution in *methanol*.

S,R,S-Enantiomer (if present). Determine by liquid chromatography (2.4.14).

Perform this test if this impurity is possible from the manufacturing process.

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of mobile phase.

Reference solution. A 0.008 per cent w/v solution of *aprepitant IPRS* and 0.008 per cent w/v solution of *aprepitant related compound B IPRS* (*S,R,S*-Enantiomer: 3-[[(2*S*,3*R*)-2-[(*S*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl) morpholino]methyl]-1*H*-1,2,4-triazol-5(4*H*)-one *IPRS*) in mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with amylose tris-3,5-dimethylphenylcarbamate coated to porous spherical silica particles (5-10 µm),
- mobile phase: 90 volumes of *hexane* and 10 volumes of *ethanol*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the enantiomer peaks is more than 2.0. (The elution order is the S,R,S-enantiomer followed by aprepitant peak, which is R,S,R-enantiomer).

Inject the test solution. The area of S,R,S-enantiomer peaks are not more than 0.10 per cent, calculated by area normalization.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *dilute orthophosphoric acid* prepared by dissolving 1 ml of *orthophosphoric acid* in 1000 ml of *water*.

Test solution. Dissolve 0.2 g of the substance under examination in 100.0 ml of the solvent mixture and sonicate.

Reference solution (a). A 0.0003 per cent w/v solution of *aprepitant IPRS* in solvent mixture.

Reference solution (b). Dilute reference solution (a) to obtain a 0.0001 per cent w/v solution of *aprepitant IPRS* in solvent mixture.

Reference solution (c). A 0.2 per cent w/v solution of *aprepitant IPRS* and 0.0003 per cent w/v solution of *desfluoro aprepitant IPRS* (*5-[[(2R ,3S)-2-[(R)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-phenylmorpholino] methyl]-2H -1,2,4-triazol-3(4H)-one IPRS*) in solvent mixture and sonicate.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: A. a solution prepared by dissolving 1 ml of *orthophosphoric acid* in 1000 ml of *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	58	42
25	58	42
45	30	70
50	30	70
50.1	58	42
55	58	42

Name	Relative retention time
Desfluoro aprepitant	0.85
Aprepitant	1.0

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to desfluoro aprepitant and aprepitant is not less than 3.0, signal to noise ratio for the principal peak is not less than 10.0 with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of the peak due to desfluoro aprepitant is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent) and the area of any other secondary peak is not more than 0.67 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak with an area less than 0.33 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (2.4.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *dilute orthophosphoric acid* prepared by dissolving 1 ml of *orthophosphoric acid* in 1000 ml of *water*.

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of solvent mixture and sonicate.

Reference solution. A 0.02 per cent w/v solution of *aprepitant IPRS* in solvent mixture and sonicate.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: 48 volumes of *acetonitrile* and 52 volumes of *dilute orthophosphoric acid*, prepared by dissolving 1 ml of *orthophosphoric* acid in 1000 ml of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 0.73 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{21}F_7N_4O_3$.

Storage. Store protected from moisture and light, at a temperature not exceeding 30°.

Aprepitant Capsules

Aprepitant Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aprepitant, $C_{23}H_{21}F_7N_4O_3$.

Usual strengths. 40 mg; 80 mg; 125 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with that of reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 2.2 per cent w/v solution of *sodium dodecyl sulphate* in *water*,
Speed and time. 100 rpm and 20 minutes.
Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *aprepitant IPRS* in minimum quantity of *methanol* and fruther dilute with dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: equal volumes of *acetonitrile* and *dilute orthophosphoric acid* prepared by dissolving 1 ml of *orthophosphoric acid* in 1000 ml of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent

Inject the reference solution and the test solution.

Q. Not less than 80 per cent of the stated amount of $C_{23}H_{21}F_7N_4O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *dilute orthophosphoric acid* prepared by dissolving 1 ml of *orthophosphoric acid* in 1000 ml of *water*.

Test solution. Disperse a quantity of the mixed contents of 20 capsules containing 0.12 g of Aprepitant in 150 ml of solvent mixture, sonicate for about 10 minutes with intermittent shaking, cool and dilute to 200.0 ml with solvent mixture. Filterthrough a 0.45 µm nylon filter.

Reference solution(a). A 0.00012 per cent w/v solution of *aprepitant IPRS* in solvent mixture.

Referencesolution(b). A 0.06 per cent w/v solution of *aprepitant IPRS* and 0.00012 per cent w/v solution of *desfluoro aprepitant IPRS (5-[[(2R,3S)-2-[(R)-1-[3,5-Bis(trifluoro-methyl)phenyl]ethoxy]-3-phenylmorpholino] methyl]-2H -1,2,4-triazol-3(4H)-one IPRS*) in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: A. a mixture of 5 volumes of *acetonitrile* and 95 volumes of *dilute orthophosphoric acid*, prepared by dissolving 1 ml of *orthophosphoric* acid in 1000 ml of *water*,

  B. a mixture of 95 volumes of *acetonitrile* and 5 volumes of *dilute orthophosphoric acid*, prepared by dissolving 1 ml of *orthophosphoric* acid in 1000 ml of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
20	58	42
25	35	65
33	35	65
33.1	60	40
38	60	40

Name	Relative retention time
Desfluoro aprepitant	0.85
Aprepitant	1.0
Aprepitant diastereomers (R,R,R and R,S,S)	1.3

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to desfluoro aprepitant and aprepitant is not less than 3.0 with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the mixed contents of 20 capsules containing 100 mg of Aprepitant in 75 ml of mobile phase, sonicate for about 20 minutes with intermittent shaking, cool and dilute to 100 ml with the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase. Filter through a 0.45 µm nylon filter.

Reference solution. A 0.005 per cent w/v solution of *aprepitant IPRS* in mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: 45 volumes of *acetonitrile* and 55 volumes of *dilute orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

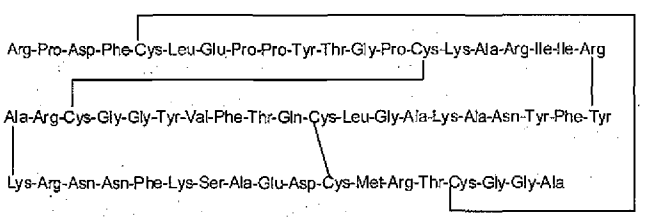
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{21}F_7N_4O_3$ in the capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Aprotinin



$C_{284}H_{432}N_{84}O_{79}S_7$ Mol. Wt. 6511.4

Aprotinin is a polypeptide consisting of a chain of 58 amino acids. It inhibits stoichiometrically the activity of several proteolytic enzymes such as chymotrypsin, kallikrein, plasmin and trypsin.

Aprotinin contains not less than 3.0 IU of aprotinin activity per mg, calculated on the dried basis.

Category. Antifibrinolytic.

Production

The animals from which aprotinin is derived must fulfil the requirements for the health of animals suitable for human consumption. The method of manufacture is validated to demonstrate that the product if tested, would comply with the following tests.

Description. An almost white hygroscopic powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *water*, 100 volumes of *glacial acetic acid* containing 10 per cent w/v of *sodium acetate*.

Solution A. Prepare a solution of the substance under examination containing 15 IU per ml, calculated from the activity stated on the label.

Test solution. Use solution A.

Reference solution. Dilute *aprotinin solution* in *water* to obtain a concentration of 15 IU per ml.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and spray with a solution of 0.1 g of *ninhydrin* in a mixture of 6 ml of a 1.0 per cent w/v solution of *cupric chloride*, 21 ml of *glacial acetic acid* and 70 ml of *ethanol (95 per cent)*. Dry the plate at 60°. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

B. Determine the ability of the substance under examination to inhibit trypsin activity using the following method.

Test solution. Dilute 1 ml of solution A to 50 ml with *buffer solution pH 7.2*.

Trypsin solution. Dissolve 10 mg of *trypsin* in *0.002 M hydrochloric acid* and dilute to 100.0 ml with *0.002 M hydrochloric acid*.

Casein solution. Dissolve 0.2 g of *casein* in *buffer solution pH 7.2* and dilute to 100.0 ml with *buffer solution pH 7.2*.

Precipitating solution. A mixture of 1 volume of *glacial acetic acid*, 49 volumes of *water* and 50 volumes of *ethanol (95 per cent)*.

Mix 1 ml of the test solution with 1 ml of the trypsin solution. Allow to stand for 10 minutes and add 1 ml of the casein solution. Incubate at 35° for 30 minutes. Cool in iced *water* and add 0.5 ml of the precipitating solution. Shake and allow to stand at room temperature for 15 minutes. The solution is cloudy. Carry out a blank test under the same conditions using *buffer solution pH 7.2* instead of the test solution. The solution is not cloudy.

Tests

Appearance of solution. Solution A is clear (2.4.1).

Absorbance. Not more than 0.8 at the absorption maxima at 277 nm (2.4.7), determined on a solution of the substance under examination containing 3.0 IU per ml.

Histamine (2.2.7). Not more than 0.2 µg of histamine base per 3 IU.

Des-Ala-aprotinin and des-Ala.-des-Gly-aprotinin. Determine by capillary zone electrophoresis (2.4.32).

Test solution. Prepare a solution of the substance under examination in *water* containing not less than 1 IU per ml.

Reference solution. Dilute *aprotinin solution IPRS* in *water* to obtain the same concentration as the test solution.

Chromatographic system

- a capillary column 45 to 60 cm x 75 µm, packed with uncoated fused silica,
- temperature: 25°,
- CZE buffer: dissolve 8.21 g of *potassium dihydrogen phosphate* in 400 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, dilute to 500.0 ml with *water*,
- spectrophotometer set at 214 nm,
- injection: under pressure or vacuum,
- migration: apply field strength of 0.2 kV/cm.

For identification of impurities, use the electropherogram supplied with aprotinin solution and the electropherogram obtained with the reference solution to identify the peaks corresponding to aprotinin impurities A and B.

The relative migration time with reference to aprotinin (migration time: about 22 minutes) for aprotinin impurity A is about 0.98 and for aprotinin impurity B is about 0.99.

Between-run, rinse the capillary for at least 1 minute with filtered *0.1 M sodium hydroxide* and for 2 minutes with the CZE buffer.

Inject the reference solution. Run the electropherogram for about 30 minutes. The test is not valid unless the resolution between the peaks corresponding to aprotinin impurities A and B is not less than 0.8 and between the peaks corresponding to aprotinin impurity B and aprotinin is not more than 0.5 and the height of the principal peak is not less than 1000 times the height of the baseline noise.

Inject the test solution. The area of any peak corresponding to aprotinin impurity A is not more than 8.0 per cent and the area of any peak corresponding to aprotinin impurity B is not more than 7.5 per cent, calculated by area normalization.

Pyroglutamyl-aprotinin and related compounds. Determine by liquid chromatography (2.4.14).

Test solution. Prepare a solution of the substance under examination in mobile phase A containing about 5 IU per ml.

Reference solution. Dissolve the contents of a vial of *aprotinin for system suitability IPRS* in 2.0 ml of mobile phase A.

Chromatographic system
- a stainless steel column 7.5 cm x 7.5 mm, packed with strong cation-exchange silica (10 µm),
- column temperature: 40°,
- mobile phase: A. dissolve 3.52 g of *potassium dihydrogen phosphate* and 7.26 g of *disodium hydrogen phosphate dihydrate* in 1000 ml of *water*,
  B. dissolve 3.52 g of *potassium dihydrogen phosphate*, 7.26 g of *disodium hydrogen phosphate dihydrate* and 66.07 g of *ammonium sulphate* in 1000 ml of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 40 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	92	8
21	64	36
30	0	100
32	92	8

The relative retention time with reference to aprotinin (retention time: about 17 to 20 minutes) for aprotinin impurity C (pyroglutamaylaprotinin) is about 0.9.

Inject the reference solution. The test is not valid unless the resolution between the peaks corresponding to aprotinin impurity C and aprotinin is not less than 1.5 and the tailing factor is not more than 2.0 for the principal peak.

Inject the test solution. The area of the peak corresponding to aprotinin impurity C is not more than 1.0 per cent. The area of any other secondary peak is not more than 0.5 per cent. The sum of areas of all the secondary peaks other than aprotinin impurity C is not more than 1.0 per cent, calculated by area normalization.

Aprotinin oligomers. Determine by size-exclusion chromatography (2.4.16).

Test solution. Prepare a solution of the substance under examination in *water* containing about 5 IU per ml.

Reference solution. Treat the substance under examination to obtain about 2.0 per cent aprotinin oligomers. (heat freeze-dried aprotinin at about 110° for about 4 hours, then dissolve in *water* to obtain a concentration of about 5 IU per ml).

Chromatographic system
- 3 column coupled in series 30 cm x 7.8 mm, packed with hydrophilic silica gel of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 20 000 to 10 000 000 (8 µm),
- mobile phase: a mixture of 20 volumes of *acetonitrile*, 20 volumes of *glacial acetic acid* and 60 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume: 100 µl.

The relative retention time with reference to aprotinin monomer (retention time: about 25 minutes) for aprotinin dimer is about 0.9.

Inject the reference solution. The test is not valid unless the resolution between the peaks corresponding to aprotinin dimer and monomer is not less than 1.3 and the tailing factor of the peak corresponding to aprotinin monomer is not more than 2.5.

Inject the test solution. Run the chromatogram for about 40 minutes. The sum of all the oiligomers is not more than 1.0 per cent, calculated by area normalization.

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 0.1 g by drying in vacuum.

Assay. The estimated activity is not less than 90 per cent and not more than 110 per cent of the activity stated on the label.

The inhibiting activity of aprotinin is expressed in IU. 1 IU inhibits 50 per cent of the enzymatic activity of 2 microkatals of trypsin. The activity of aprotinin is determined by measuring its inhibitory action on a solution of trypsin of known activity. The inhibiting activity of the aprotinin is calculated from the difference between the initial activity and the residual activity of the trypsin.

Use a reaction vessel with a capacity of about 30 ml, provided with the following options:
- device that will maintain a temperature of 25°,
- a stirring device (Such as magnetic stirrer),
- a lid with 5 holes for accommodating the electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of the reagents.

An automatic or manual titration apparatus may be used. In the latter case the burette is graduated in 0.05 ml and the pH meter is provided with a wide reading scale and glass and calomel or glass-silver-silver chloride electrodes.

Test solution. Prepare a solution of the substance under examination in *0.0015 M borate buffer solution pH 8.0* to contain 1.67 IU per ml (about 0.6 mg (*m* mg) per ml).

Trypsin solution. Prepare a solution of *trypsin* containing about 0.8 microkatals per ml (about 1 mg per ml), using *0.001 M*

hydrochloric acid. (Use a freshly prepared solution and keep in iced water).

Trypsin and aprotinin solution. To 4.0 ml of the trypsin solution, add 1.0 ml of the test solution. Dilute immediately to 40.0 ml with *0.0015 M borate buffer solution pH 8.0*. (Allow to stand at room temperature for 10 minutes and then keep in iced water. Use within 6 hours of preparation).

Dilute trypsin solution. Dilute 0.5 ml of the trypsin solution to 10.0 ml with *0.0015 M borate buffer solution pH 8.0*. (Allow to stand at room temperature for 10 minutes and then keep in iced water).

Maintain an atmosphere of nitrogen in the reaction flask and stir continuously; introduce 9.0 ml of *0.0015 M borate buffer solution pH 8.0* and 1.0 ml of a freshly prepared 0.69 per cent w/v solution of *benzoylarginine ethyl ester hydrochloride*, adjusted to pH 8.0 with *0.1 M sodium hydroxide*. When the temperature has reached equilibrium at 25°, add 1.0 ml of the trypsin and aprotinin solution and start a timer. Maintain at pH 8.0 by the addition of *0.1 M sodium hydroxide* and note the volume added every 30 seconds. Continue the reaction for 6 minutes. Determine the number of ml of 0.1 M sodium hydroxide used per second (n_1 ml). Carry out, under the same conditions, a titration using 1.0 ml of the dilute trypsin solution. Determine the number of ml of *0.1 M sodium hydroxide* used per second (n_2 ml).

Calculate the aprotinin activity in IU per mg using the following expression:

$$\frac{4000\,(2n_2 - n_1)}{m}$$

If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

Bacterial endotoxins (2.2.3). Not more than 0.14 Endotoxin Units per IU of aprotinin.

Storage. Store protected from light and moisture.

Labelling. The label states the number of IU of aprotinin activity per mg and where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Aprotinin Injection

Aprotinin Injection is a sterile solution of Aprotinin in Water for Injection that also contains Sodium Chloride. One Aprotinin Unit is equivalent to 1800 Kallikrein Inhibition Units (K.I.U.)

Aprotinin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aprotinin, $C_{284}H_{432}N_{84}O_{79}S_7$, expressed in K.I.U. per ml.

Usual strength. 20000 K.I.U. per ml.

Identification

In the limit of *N*-Pyroglutamyl-Aprotinin and Related compounds, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 6.5.

Limit of *N*-Pyroglutamyl-Aprotinin and Related compounds. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the injection with mobile phase A to obtain a solution containing 5 Aprotinin units per ml.

Reference solution. Dissolve a suitable quantity of *aprotinin system suitability IPRS* in mobile phase A to obtained a solution containing 5 Aprotinin units per ml.

Chromatographic system

- a stainless steel column 7.5 cm x 7.5 mm, packed with a strong cation exchange resin with sulfopropyl groups bonded to porous silica (5 µm) (Such as Supelcosil LC-SCX),
- column temperature: 40°,
- mobile phase: A. a buffer solution prepared by dissolving 3.52 g of *monobasic potassium phosphate* and 7.26 g of *dibasic sodium phosphate* in 1000 ml of *water*,

  B. a buffer solution prepared by dissolving 3.52 g of *monobasic potassium phosphate*, 7.26 g of *dibasic sodium phosphate* and 66.07 g of *ammonium sulphate* in 1000 ml of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 40 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	92	8
21	64	36
30	0	100
31	92	8
40	92	8

The relative retention time with reference to aprotinin for *N*-pyroglutamyl-aprotinin is about 0.9.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to *N*-pyroglutamyl-aprotinin and aprotinin is not less than 1.0 and the tailing factor is not more than 2.0 for aprotinin peak.

Inject the test solution. The area of any peak corresponding to *N*-pyroglutamyl-aprotinin is not more than 1.0 per cent, the area of any other secondary peak is not more than 0.5 per cent and the sum of the areas of all the secondary peaks other than *N*-pyroglutamyl-aprotinin, is not more than 1.0 per cent, calculated by area normalization.

High molecular weight proteins. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the injection in *water* to obtain a solution containing 5 Aprotinin Units per ml.

Reference solution. A solution containing 5 Aprotinin Units per ml with about 2 per cent w/v of aprotinin oligomers. *(NOTE — This solution can be obtained by heating lyophilized aprotinin at 112° for about 2 hours and dissolving the solid at the specified concentration in water).*

Chromatographic system

- a series of three stainless steel column 30 cm x 7.8 mm, packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 Da (Such as TSK gel Ultra SW Aggregate),
- mobile phase: a mixture of 20 volumes of *acetonitrile*, 20 volumes of *glacial acetic acid* and 60 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 100 µl.

The relative retention times with reference to aprotinin for dimer is about 0.9.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to dimer and aprotinin is not less than 1.3 and the tailing factor is not more than 2.5 for aprotinin peak.

Inject the test solution. The sum of the areas of all the peaks with retention time less than that of aprotinin monomer is not more than 1.5 per cent, calculated by area normalization.

Content of sodium chloride. 42.5 to 47.5 mg.

Dilute 5.0 ml of the injection with 50.0 ml with *water*. Add 10 ml of 25 per cent v/v of *nitric acid*. Titrate with *0.1 M silver nitrate*, determining the endpoint potentiometrically (2.4.25. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 0.14 Endotoxin Units per aprotinin unit.

Sterility (2.2.11). Complies with the test for sterility.

Assay.

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Dissolve 0.93 g of *boric acid* in 900 ml of *water*, adjusted to pH 8.0 with *sodium hydroxide* and dilute to 1000.0 ml with *water*. Dilute 10.0 ml of the solution to 100.0 ml with *water*.

Test solution. Dilute a suitable volume of the injection with the solvent mixture to obtain a solution containing 1.67 Aprotinin Units per ml.

Reference solution (a). A solution containing 4300 Trypsin Units per ml of *trypsin crystallized IPRS* in *0.001M hydrochloric acid*. Use a freshly prepared solution, and store in ice-water.

Reference solution (b). Dilute 4.0 ml of reference solution (a) and 1.0 ml of the test solution to 40.0 ml with the solvent mixture. Allow to stand the solution at room temperature for 10 minutes and then keep in ice-water. Use the solution within 6 hours of preparation.

Reference solution (c). Dilute 0.5 ml of reference solution (a) to 10.0 ml with the solvent mixture. Allow to stand the solution at room temperature for 10 minutes then store in ice-water.

Reference solution (d). A 0.69 per cent w/v solution of *N-benzoyl-L-arginine ethyl ester hydrochloride* in the solvent mixture. Use the solution within 2 hours.

Dilute 1.0 ml of reference solution (d) with 9.0 ml of the solvent mixture in a jacketed-glass vessel with a capacity of about 30 ml and containing a stirring device. The lid of the reaction vessel should contain five holes to accommodate the electrodes, the tip of a burette, a tube for the admission of nitrogen, and the introduction of reactants. An automated or manual titration apparatus may be used. Adjusted to pH 8.0 with *0.1M sodium hydroxide*. Maintain an atmosphere of nitrogen within the vessel, and stir continuously. When the temperature has reached equilibrium at 25 ± 0.1°, add 1.0 ml of reference solution (b), and start a timer. Maintain at a pH of 8.0 by the addition of *0.1 M sodium hydroxide*, and record the volume added every 30 seconds. Continue the reaction for 6 minutes. Carry out a similar titration using 1.0 ml of reference solution (c).

Calculate the potency in Aprotinin units per ml by using following expression.

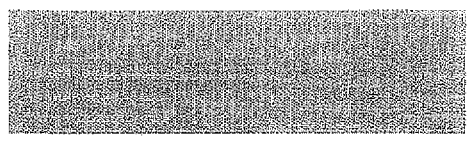
$$\text{Potency (Aprotinin units per ml)} = C_1 \times (C_2 \times V_2 - V_1) \times D$$

where, C_1 = conversion factor, 4000;

C_2 = difference in the amount of trypsin used in reference solution (b) and reference solution (c), 2;

V_1 = volume of *0.1 M sodium hydroxide* added per second, after adding reference solution (b) (ml per second);

V_2 = volume of *0.1 M sodium hydroxide* added per second, after adding reference solution (c) (ml per second);

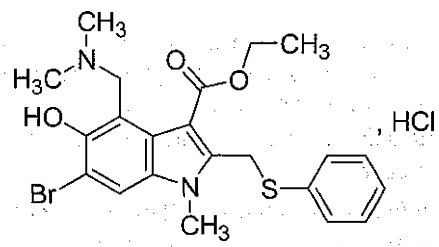


D = dilution factor used to prepare the test solution.

Storage. Store protected from moisture, in a single dose containers, at a temperature not exceeding 25°, do not freeze.

Arbidol Hydrochloride

Arbidol Hydrochloride Monohydrate



$C_{22}H_{25}BrN_2O_3S$, HCl, H_2O Mol Wt. 531.9

Arbidol Hydrochloride is Ethyl 6-bromo-5-hydroxy-4-[(dimethylamino)methyl]-1-methyl-2-[(phenylthio)methyl]-1*H*-indole-3-carboxylate hydrochloride.

Arbidol Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{25}BrN_2O_3S$, HCl, calculated on the anhydrous basis.

Category. Antiviral.

Description. An off white to yellow colour powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *arbidol hydrochloride monohydrate IPRS* or with the reference spectrum of arbidol hydrochloride monohydrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes *acetonitrile* and 10 volumes of *water.*

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with solvent mixture.

Reference solution. A 0.001 per cent w/v solution of *arbidol hydrochloride monohydrate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- sample temperature: 5°,
- mobile phase: A. dissolve 1.36 g of *potassium dihydrogen orthophosphate monohydrate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, B. a mixture of 90 volumes of *acetonitrile* and 10 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0.01	55	45
15	45	55
25	25	75
50	10	90
55	55	45
60	55	45

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Chlorides. 6.5 per cent to 8.5 per cent.

Dissolve 0.1 g in 100 ml of *methanol* and titrate with *0.1 M silver nitrate*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of chloride.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 3.0 per cent to 4.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in *acetonitrile* and dilute to 50.0 ml with *acetonitrile.*

Reference solution. A 0.02 per cent w/v solution of *arbidol hydrochloride monohydrate IPRS* in *acetonitrile.*

Chromatographic system
- a stainless steel column 15cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- sample temperature: 5°,
- mobile phase: A. dissolve 1.36 g of *potassium dihydrogen orthophosphate monohydrate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, B. a mixture of 90 volumes of *acetonitrile* and 10 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0.01	55	45
8	55	45
20	10	90
25	10	90
30	55	45
35	55	45

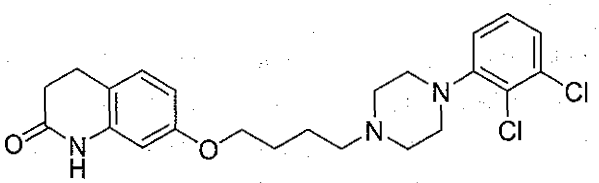
Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates., the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{25}BrN_2O_3S$, HCl.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Aripiprazole



$C_{23}H_{27}Cl_2N_3O_2$ Mol. Wt. 448.4

Aripiprazole is 7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one.

Aripiprazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{23}H_{27}Cl_2N_3O_2$, calculated on the anhydrous basis.

Category. Antipsychotic; Neuroleptic.

Description. A white to light yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aripiprazole IPRS* or with the reference spectrum of aripiprazole.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, add 2 ml of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid*, 25 volumes of *methanol* and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the test solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 80 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase. Further dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.0004 per cent w/v solution of *aripiprazole IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{27}Cl_2N_3O_2$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Aripiprazole Tablets

Aripiprazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of aripiprazole, $C_{23}H_{27}Cl_2N_3O_2$.

Usual strengths. 5 mg; 10 mg; 15 mg; 20 mg; 30 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 1000 ml of *0.1M hydrochloric acid*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium.

Determine by liquid chromatography (2.4.14).

Test solution. Centrifuge the medium at 3500 rpm for 15 minutes and use the supernatant solution. Dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *aripiprazole IPRS* in *acetonitrile* and dilute with the dissolution medium to obtain a solution of the same concentration as that of the test solution.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{27}Cl_2N_3O_2$ in the medium.

Q. Not less than 65 per cent of the stated amount of $C_{23}H_{27}Cl_2N_3O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 10 tablets. Disperse a quantity of powder containing 25 mg of Aripiprazole in 70 ml of the mobile phase with the aid of ultrasound for 45 minutes and dilute to 100.0 ml with the mobile phase. Centrifuge the solution at 3500 rpm for 15 minutes. Dilute 5.0 ml of the supernatant liquid to 25.0 ml with the mobile phase.

Reference solution. A 0.003 per cent w/v solution of *aripiprazole IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as YMC ODS),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, add 2 ml of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid*, 25 volumes of *acetonitrile* and 25 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 1.0 per cent and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization method.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions as described under Assay.

Test solution. Disperse one tablet in 10 ml of *acetonitrile* with the aid of ultrasound for 20 minutes. Add 80 ml of the mobile phase in the solution, further sonicate for 50 minutes and dilute to 100.0 ml with the mobile phase. Centrifuge the solution at 3500 rpm for 15 minutes. Dilute 5.0 ml of the supernatant liquid to 50.0 ml with the mobile phase.

Reference solution. Dissolve a quantity of *aripiprazole IPRS* in *acetonitrile* and dilute with the mobile phase to obtain a solution of the same concentration as that of the test solution.

Calculate the content of $C_{23}H_{27}Cl_2N_3O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 30 mg of Aripiprazole in 10 ml of *acetonitrile* with the aid of ultrasound for 20 minutes. Add 75 ml of the mobile phase in the solution, further sonicate for 40 minutes and dilute to 100.0 ml with the mobile phase. Centrifuge this solution at 3500 rpm for 15 minutes. Dilute 5.0 ml of the supernatant liquid to 50.0 ml with the mobile phase.

Reference solution. Dissolve 30 mg of Aripiprazole in 10 ml of *acetonitrile* with the aid of ultrasound and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as YMC ODS),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, add 2 ml of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid*, 25 volumes of *acetonitrile* and 25 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 20 µl.

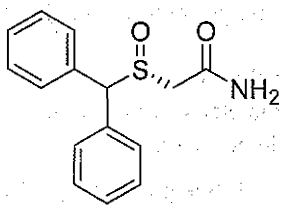
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{27}Cl_2N_3O_2$ in the tablets.

Storage. Store protected from light and moisture.

Armodafinil



$C_{15}H_{15}NO_2S$ Mol. Wt. 273.4

Armodafinil is 2-[(*R*)-(diphenylmethyl)sulfinyl]acetamide.

Armodafinil contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{15}NO_2S$, calculated on the dried basis.

Category. Wakefulness-promoting agent.

Description. A white to off white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *armodafinil IPRS* or with the reference spectrum of armodafinil.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Dissolve 50 mg of the substance under examination and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.01 per cent w/v solution of *armodafinil IPRS* in solvent mixture.

Reference solution (b). A 0.0075 per cent w/v solution containing each of *armodafinil impurity A IPRS, armodafinil impurity B IPRS, armodafinil impurity C IPRS and armodafinil impurity D IPRS* in solvent mixture.

Reference solution (c). Dilute 2.0 ml of reference solution (b) and 1.0 ml of reference solution (a) in 100.0 ml of solvent mixture.

Reference solution (d). Dissolve 50 mg of *armodafinil IPRS* in about 30 ml of solvent mixture, add 1.0 ml of reference solution (b), and dilute to 50.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as X-Terra C18),
- mobile phase: A. dissolve 2.72 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 4.0 with *dilute orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
10	75	25
20	35	65
35	35	65
38	75	25
45	75	25

Name	Relative retention time
Impurity A[1]	0.5
Armodafinil (Retention time: about 8 minutes)	1
Impurity B[2]	2.0
Impurity C[3]	2.3
Impurity D[4]	2.4

[1](*R*)-(-)-(Diphenylmethanesulfinyl) acetic acid,

[2]2-(Benzhydrylsulfonyl) acetamide,

[3](*R*)-(-)-Methyl(Diphenylmethanesulfinyl) acetate,

[4]2-(Benzhydrylsulfanyl) acetamide.

Inject reference solution (c). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent for armodafinil peak.

Inject reference solution (d) and the test solution. In the chromatogram obtained with test solution, the area of any secondary peak is not more than 0.15 per cent, calculated by area normalization. The test is not valid unless the resolution between the peaks due to armodafinil impurity C and armodafinil impurity D is not less than 1.5, the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0 for armodafinil peak.

2-[(S)-(diphenylmethyl)sulfinyl]acetamide (S-isomer). Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 100.0 ml of mobile phase.

Reference solution (a). Dissolve 1 mg of *S*-isomer in 10.0 ml of the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *armodafinil IPRS* in sufficient mobile phase and add 1.0 ml of reference solution (a) and dilute to 100.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm, packed with immobilised α-1 acid glycoprotein on spherical silica particles (Such as Chiral-AGP) (5µm),
- mobile phase; dissolve 3.9 g of *ammonium acetate* in 1000 ml of *water* and add 8 ml of *1-butanol*, adjusted to pH 6.75 with *sodium hydroxide solution* or *dilute acetic acid*,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Name	Relative retention time
Armodafinil (Retention time: about 7.2 minutes)	1
S-isomer	1.3

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to *S*-isomer and armodafinil is not less than 1.5 and the related standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to *S*-isomer is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in oven at 105° for 2 hours.

Assay. Determine by liquid chromatography (2.4.14)

Solvent mixture. A mixture of equal volumes of mobile phase A and mobile phase B.

Test solution. Dissolve 50 mg of the substance under examination and dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *armodafinil IPRS* in solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as X-Terra C18),
- mobile phase: A. dissolve 2.72 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 4.0 with *dilute orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
10	75	25
20	35	65
25	35	65
26	75	25
30	75	25

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

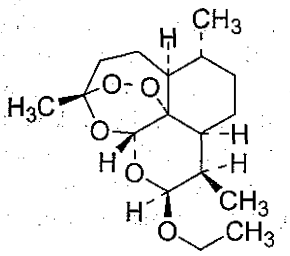
Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{15}NO_2S$.

Storage. Store protected from moisture.

Arteether

α-β Arteether



$C_{17}H_{28}O_5$ Mol. Wt. 312.4

α-arteether is (3*R*,5a*S*,6*R*,10*R*,12*S*,12a*R*)-10-Ethoxydecahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin.

β-arteether is (3*R*,5a*S*,6*R*,10*S*,12*S*,12a*R*)-10-Ethoxydecahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin.

Arteether contains α-isomer not less than 25.0 per cent and not more than 35.0 per cent and β-isomer not less than 65.0 per cent and not more than 75.0 per cent and total arteether is not less than 95.0 per cent and not more than 105.0 per cent of $C_{17}H_{28}O_5$, calculated on the dried basis.

Category. Antimalarial.

Description. A light yellow coloured lipophilic semi-solid.

Identification

Test A may be omitted if test B is carried out. Test B may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *arteether IPRS* or with the reference spectrum of arteether.

B. In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *hexane* is clear (2.4.1).

Specific optical rotation (2.4.22). + 100.0° to + 120.0°, at 20°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *hexane* and 10 volume of *ethyl acetate*.

Test solution. Dissolve 0.5 g of the substance under examination in 10.0 ml of *chloroform*.

Reference solution (a). A 0.15 per cent w/v solution of *arteether IPRS* in *chloroform*.

Reference solution (b). A 0.10 per cent w/v solution of β-*arteether IPRS* in *chloroform*.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 10.0 ml with *chloroform*.

Apply to the plate 6 µl of each solution. After development, dry the plate at 60° for 15 minutes. Spray with a 4 per cent w/v solution of *vanillin* in *sulphuric acid* and examine in daylight. Any spot in the chromatogram obtained with the test solution other than the principal spots is not more intense than the spot in the chromatogram obtained with reference solution (b). Not more than one such spot is more intense than that in the chromatogram obtained with reference solution (c).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 2 per cent, determined on 1.0 g at 35° under vacuum for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.15 g of the substance under examination in 100.0 ml of *acetonitrile*.

Reference solution. A 0.15 per cent w/v solution of *arteether IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

The relative retention time with respect to β-arteether, for α-arteether is about 0.7.

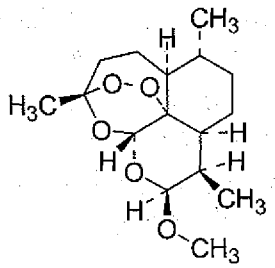
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of total arteether, $C_{17}H_{28}O_5$, and of the α- and β-isomers.

Storage. Store protected from light and moisture.

Artemether



$C_{16}H_{26}O_5$ Mol. Wt. 298.4

Artemether is (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin.

Artemether contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{16}H_{26}O_5$, calculated on the dried basis.

Category. Antimalarial.

Description. A white crystals or a white crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *artemether IPRS* or with the reference spectrum of artemether.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Dissolve 30 mg in 1 ml of *anhydrous ethanol* and add 0.1 g of *potassium iodide*. Heat the mixture on a water-bath. A yellow colour is produced.

D. Dissolve 30 mg in 6 ml of *anhydrous ethanol*. Add a few drops on a white porcelain dish and add 1 drop of *vanillin sulphuric acid TS*. A pink colour is produced.

Tests

Specific optical rotation (2.4.22). +166.0° to +173.0° at 20°, determined in a 1.0 per cent w/v solution in *anhydrous ethanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of the mobile phase.

Reference solution. A 0.005 per cent w/v solution of the substance under examination in the mobile phase.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The area of not more than one such peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.25 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorous pentaoxide* under vacuum at 2.67 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in the mobile phase and dilute to 10.0 ml of the mobile phase.

Reference solution. A 1.0 per cent w/v solution of *artemether IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 62 volumes of *acetonitrile*, 38 volumes of *water*,
- flow rate:1.5 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{26}O_5$.

Storage. Store protected from light and moisture.

Artemether and Lumefantrine Tablets

Artemether and Lumefantrine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of artemether, $C_{16}H_{26}O_5$ and lumefantrine, $C_{30}H_{32}Cl_3NO$.

Usual strengths. 20 mg Artemether and 120 mg Lumefantrine; 40 mg Artemether and 240 mg Lumefantrine; 60 mg Artemether

and 360 mg Lumefantrine; 80 mg Artemether and 480 mg Lumefantrine

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2)

For Artemether —

Apparatus No. 2 (Paddle),

Medium. 1000 ml of a buffer solution prepared by dissolving 1.4 g of *disodium hydrogen phosphate anhydrous* in 1000 ml of *water*, add 10 g of *sodium lauryl sulphate* and adjusted to pH 7.2 with *dilute hydrochloric acid,*

Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 20 mg of *artemether IPRS* in 2 ml of *acetonitrile*, with the aid of ultrasound and dilute to 100.0 ml with the dissolution medium. Dilute 1.0 ml of the solution to 10.0 ml with the dissolution medium.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3V),
- column temperatue: 40°,
- sample temperature: 10°,
- mobile phase: A. a buffer solution prepared by mixing 12 ml of *triethylamine* with 1000 ml of *water*, adjusted to pH 2.3 with *orthophosphoric acid,*
  B. a mixture of 95 volumes of *acetonitrile* and 5 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 2.0 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	27	73
9	30	70
9.5	27	73
12	27	73

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{26}O_5$ in the medium.

Q. Not less than 60 per cent of the stated amount of $C_{16}H_{26}O_5$.

For Lumefantrine —

Apparatus No. 2 (Paddle),

Medium. 1000 ml of 2 per cent v/v of *benzalkonium chloride solution* in *0.1M hydrochloric acid,*

Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.12 per cent w/v solution of *lumefantrine IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the dissolution medium.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Waters symmetry),
- sample temperature: 10°,
- mobile phase: a mixture of 25 volumes of a buffer solution prepared by dissolving 5.65 g of *sodium-1-hexane sulphonate* and 2.75 g of *sodium dihydrogen phosphate monohydrate* in 800 ml of *water*, add 5.0 ml of *triethylamine* and adjusted to pH 2.3 with *dilute orthophosphoric acid,* dilute to 1000 ml with *water* and 75 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 380 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless he relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

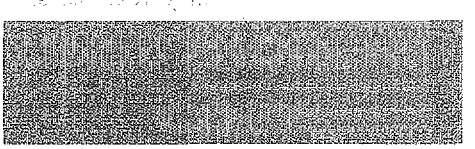
Calculate the content of $C_{30}H_{32}Cl_3NO$ in the medium.

Q. Not less than 60 per cent of the stated amount of $C_{30}H_{32}Cl_3NO$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of 0.1 per cent v/v solution of *orthophosphoric acid* and *acetonitrile.*

Test solution. Disperse a quantity of powdered tablets containing 360 mg of Lumefantrine in 15 ml of the solvent mixture, with the aid of ultrasound for 5 minutes with intermittent shaking and dilute to 25.0 ml with the solvent mixture and filter.



Reference solution (a). A 0.012 per cent w/v solution of *artemether IPRS* in the solvent mixture.

Reference solution (b). A 0.0144 per cent w/v solution of *lumefantrine IPRS* in the solvent mixture.

Reference solution (c). Dilute 25.0 ml of reference solution (a) and 5.0 ml of reference solution (b) to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS 3V),
- mobile phase: a mixture of 40 volumes of 0.1 per cent v/v solution of *orthophosphoric acid* and 60 volumes of *acetonitrile*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 25 µl.

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 5.0 per cent for both the peaks.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 5 times the area of the principal peak due to lumefantrine in the chromatogram obtained with reference solution (c) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 20 times the area of the principal peak due to lumefantrine in the chromatogram obtained with reference solution (c) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Mix 12 ml of *triethylamine* with 1000 ml of *water*, adjusted to pH 2.3 with *orthophosphoric acid.*

Solvent mixture. Dilute 20 ml of the buffer solution, 6 ml of *water,* 20 ml of *isopropyl alcohol* to 100.0 ml with *acetonitrile.*

Test solution. Transfer 5 intact tablets into 1000-ml volumetric flask, add 60 ml of *water*, 200 ml of *isopropyl alcohol* and dissolve with the aid of ultrasound for 15 minutes, add 200 ml of buffer solution and 400 ml of *acetonitrile*, dissolve with the aid of ultrasound for 45 minutes by maintaining water temperature of sonicator at 15° and dilute to volume with *accetonitrile*. Dilute a suitable volume of the solution with the solvent mixture to obtain the concentration similar to the reference solution.

Reference solution. A solution containing 0.01 per cent w/v of *artemether IPRS* and 0.06 per cent w/v of *lumefantrine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3V),
- column temperatue: 40°,
- sample temperature: 10°,
- mobile phase: A. buffer solution,
  B. a mixture of 95 volumes of *acetonitrile* and 5 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm for artemether and 380 nm for lumefantrine,
- injection volume: 30 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	27	73
9	30	70
9.5	27	73
12	27	73

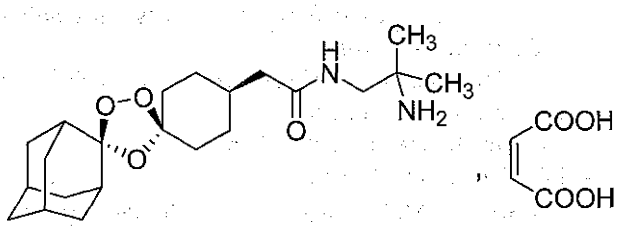
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent for lumefantrine and not more than 3.0 per cent for artemether.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{26}O_5$ and $C_{30}H_{32}Cl_3NO$ in the tablets.

Storage. Store protected from light, at a temperature not exceeding 30°.

Arterolane Maleate



$C_{26}H_{40}N_2O_8$ Mol Wt. 508.6

Arterolane Maleate is [(*N*-(2-amino-2-methylpropyl)-2-*cis*-dispiro(adamantane-2,3'-[1,2,4]trioxolane-5',1"-cyclohexane)-4"- yl]acetamide maleate.

Arterolane Maleate contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{26}H_{40}N_2O_8$, calculated on the anhydrous basis.

Category. Antimalarial.

Description. A white to off- white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *arterolane maleate IPRS* or with the reference spectrum of arterolane maleate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water.*

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Reference solution. A 0.0025 per cent w/v solution of *arterolane maleate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 1.36 g of *potassium dihydrogen orthophosphate* into 1000 ml of *water*, add 1.0 ml of *triethylamine*, adjusted to pH 4.5 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 25 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
7	90	10
20	60	40
30	20	80
40	15	85
50	15	85
55	90	10
70	90	10

Inject the reference solution. The test is not valid unless the column efficiency is not less than 10000 theoretical plates and tailing factor is not more than 3.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Maleic acid. 22.0 per cent to 24.5 per cent w/w, calculated on anhydrous basis. Weigh 0.25 g and dissolve in 10 ml of *methanol* and 70 ml of *water.* Titrate with *0.1 M sodium hydroxide,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.011607 g of maleic acid

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *arterolane maleate IPRS* in the mobile phase.

Chromatographic system

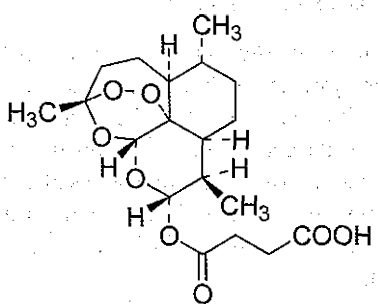
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 50 volumes of 0.2 per cent v/v *triethylamine* in *water*, and adjusted to pH 3.0, with *orthophosphoric acid* and 50 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 600 theoretical plates, the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution and the test solution.

Calculate the content of $C_{26}H_{40}N_2O_8$.

Artesunate



$C_{19}H_{28}O_8$ Mol. Wt. 384.4

Artesunate is (3*R*,5a*S*,6*R*,8a*S*,9*R*,10*R*,12*R*,12a*R*)-decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano-[4,3-*j*]-1,2-benzodioxepin-10-ol hydrogen succinate.

Artesunate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{19}H_{28}O_8$, calculated on the dried basis.

Category. Antimalarial.

Description. A white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *artesunate IPRS* or with the reference spectrum of artesunate.

Tests

pH (2.4.24). 3.5 to 4.5, determined on 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). + 4.5° to + 6.5°, determined in a 1.0 per cent w/v solution in *dichloromethane* at 20°.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in *acetonitrile* and dilute to 10.0 ml with *acetonitrile*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

Reference solution (b). A solution containing 0.1 per cent w/v of *artesunate IPRS* and 0.01 per cent w/v each of *artenimol IPRS* and *artemisinin IPRS* in *acetonitrile*.

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 56 volumes of a buffer solution prepared by dissolving 1.36 g *of potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 44 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume: 20 µl.

Name	Relative retention time
Artesunate impurity A (10-epi-artenimol)[1]	0.58
Artenimol	0.91
Artesunate (Retention time: about 9 minutes)	1.0
Artesunate impurity B (artemisinin)[2]	1.3
Artesunate impurity C (anhydrodihydroartemisinin)[3]	2.7

[1](3R,5aS,6R,8aS,9R,12R,12aR)-3,6,9-trimethyldecahydro-12*H*-3,12-epoxypyrano[4,3-j]-1,2-benzodioxepin-10-ol(dihydroartemisinins: artenimol and (10R)-artenimol),

[2](3R,5aS,6R,8aS,9R,12S,12aR)-3,6,9-trimethyloctahydro-3,12-epoxypyrano[4,3-j]-1,2-benzodioxepin-10(3*H*)-one (artemisinin),

[3](3R,5aS,6R,8aS,12R,12aR)-3,6,9-trimethyl-3,4,5,5a,6,7,8,8a-octahydro-12*H*-3,12-epoxypyrano[4,3-j]-1,2-benzodioxepine (anhydrodihydroartemisinin).

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio (Hp/Hv) is not less than 5.0, where Hp is the height above the baseline of the peak due to artenimol and Hv is the height above the baseline of the lowest point of the curve separating the peak due to artenimol from the peak due to artesunate.

Inject reference solution (a) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the combined area of any peak due to 10-epi-artenimol and artenimol is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of any peak due to impurity B (artemisinin) is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any peak due to impurity C multiplied by correction factor 0.07, is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of mobile phase and 50 volumes of *methanol.*

Test solution. Dissolve about 0.4 g of the substance under examination in 70 ml of the solvent mixture, sonicate for 15 minutes and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.4 per cent w/v solution of *artesunate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of 30 volumes of a solution containing 3.85 g of *ammonium acetate* and 1 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 5.5 with *acetic acid* and 70 volumes of *methanol*,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{28}O_8$.

Storage. Store protected from light and moisture.

Artesunate Injection

Artesunate Injection is a sterile material consisting of Artesunate with or without buffering agents and other excipients. It is filled in a sealed container.

Artesunate injection is constituted by dissolving the contents of the sealed container in the requisite amount of 5 per cent w/v sodium bicarbonate injection, shake vigorously for 5 minutes and add requisite amount of 0.9 per cent w/v sodium chloride injection, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Artesunate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of artesunate, $C_{19}H_{28}O_8$.

Usual strengths. 60 mg per vial and 120 mg per vial.

Description. A white or almost white crystalline powder.

The content of the sealed container comply with the requirements stated under Parenteral Preparations (powder for Injection) and with the following requirements.

Identification

Test A may be omitted if test B, C and D are carried out. Test B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *artesunate IPRS* or with the reference spectrum of artesunate.

B. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 70 volumes of *ethanol (95 per cent)*, 30 volumes of *toluene* and 1.5 volumes of *strong ammonium solution.*

Test solution. Dissolve a quantity of the content of the sealed container containing about 0.1 g of Artesunate in 100.0 ml of *methanol.*

Reference solution. A 0.1 per cent w/v solution of *artesunate IPRS* in *methanol.*

Apply to the plate 1 µl of each solution. After development, dry the plate in a current of warm air. Spray with *anisaldehyde methanol solution* and heat the plate at 120° for five minutes and examine in day light. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

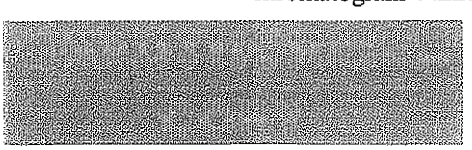
C. Dissolves a quantity of powder containing about 0.1 g of Artesunate in 40 ml of *ethanol*, shake and filter. To half of the filtrate (keep the remaining filtrate for test D), add about 0.5 ml of *hydroxylamine hydrochloride* and 0.25 ml of *2 M sodium hydroxide*. Heat the mixture in a water-bath to boiling, cool, add 2 drops of *1 M hydrochloric acid* and 2 drops of *ferric chloride test solution*; a light red violet colour is produced.

D. Evaporate the remaining filtrate on a water-bath to a volume of about 5.0 ml. place a few drops of the mixture on a white porcelain dish, add one drop of *vanillin sulphuric acid solution*, a reddish-brown colour is produced.

Tests

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Inject reference solution (b), (c) and the test solution. Run the chromatogram 4 times the retention time of artesunate.



The relative retention time with reference to artesunate for α-artenimol is about 0.58, for β-artenimol is about 0.91, for impurity B (artemisinin) is about 1.3 and for artesunate impurity C (anhydrodihydroartemisinin) is about 2.7.

Inject the reference solution (b), the test is not valid unless the peak to valley ratio (Hp/Hv) is 5.0, where Hp is the height above the baseline of the peak due to β-artenimol and Hv is the height above the baseline of the lowest point of the curve separating this peak due to artesunate.

Inject the reference solution (c) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to α-artenimol and β-artenimol (impurity A) is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any peak due to impurity B is not more than 0.5 times the area of the principal peak obtained with reference solution (c) (0.5 per cent), the area of any peak due to impurity C multiplied by correction factor of 0.07, is not more than 0.3 times the area of the principal peak obtained with reference solution (c) (0.3 per cent) and the area of any other secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent). The sum of the areas of all the secondary peaks including impurity C is not more than twice the area of the principal peak obtained with reference solution (c) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Bacterial endotoxins (2.2.3). Not more than 2.5 Endotoxin Units per mg of artesunate.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Determine the weight of the content of 10 containers. Transfer a weighed quantity of the mixed content of the 10 containers containing 40 mg of Artesunate in to a 10.0 ml volumetric flask, add about 7 ml of *acetonitrile* and dilute to volume with same solvent and filter.

Reference solution (a). A 0.4 per cent w/v solution of *artesunate IPRS* in *acetonitrile*.

Reference solution (b). A solution containing 0.01 per cent w/v solution of *artenimol IPRS*, 0.01 per cent w/v of *artemisinin IPRS* and 0.1 per cent w/v of *artesunate IPRS* in *acetonitrile*.

Reference solution (c). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

Chromatographic systems

- a stainless column 10 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (3μm),
- mobile phase: a mixture of 44 volumes of *acetonitrile* and 56 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume: 20 μl.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 4 times the retention time of artesunate.

The relative retention time with reference to artesunate for α-artenimol is about 0.58, for β-artenimol is about 0.91 and for artemisinin (artesunate impurity b) is about 1.3.

Inject reference solution (b) and the test solution. The test is not valid unless the peak-to-valley ratio (Hp/Hv) is not less than 5.0, where Hp is the height above the baseline of the peak due to β-artenimol and Hv is the height above the baseline of the lowest point of the curve separating this peak due to artesunate. The chromatogram obtained with test solution may show a peak due to impurity C eluting at a relative retention time of about 2.7 with reference to artesunate.

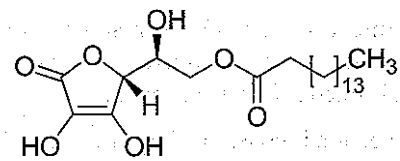
Inject reference solution (a) and the test solution.

Calculate the content of $C_{19}H_{28}O_8$ In the injection.

Storage. Store protected from moisture.

Labelling. The label states (1) the direction for constituting the solution; (2) the name of any added buffering agent or pharmaceutical aids.

Ascorbyl Palmitate



$C_{22}H_{38}O_7$ Mol. Wt. 414.5

Ascorbyl Palmitate is (2*S*)-2-[(2*R*)-3,4-Dihydroxy-5(2*H*)-oxo-2-furyl]-2-hydroxyethyl hexadecanoate.

Ascorbyl Palmitate contains not less than 95.0 per cent and not more than 100.5 per cent of $C_{22}H_{38}O_7$, calculated on the dried basis.

Category. Pharmaceutical aid.

Description. A white or yellowish-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ascorbyl*

palmitate IPRS or with the reference spectrum of ascorbyl palmitate.

Tests

Specific optical rotation (2.4.22). +21° to +24°, determined on 10.0 per cent w/v solution in *methanol*.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

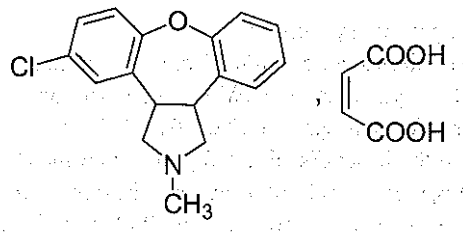
Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying under vacuum at 60° for 1 hours.

Assay. Weigh 0.16 g and dissolve in 50 ml of *methanol*. Add 30 ml of *water* and 1 ml of *starch solution*. Titrate with *0.05 M iodine* until a persistent violet-blue colour is obtained.

1 ml of *0.05 M iodine* is equivalent to 0.02073 g of $C_{22}H_{38}O_7$.

Storage. Store protected from light and moisture.

Asenapine Maleate



$C_{21}H_{20}ClNO_5$ Mol Wt.401.8

Asenapine Maleate is 5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1*H*-dibenz[2,3:6,7]oxepino[4,5-c]pyrrole maleate.

Asenapine Maleate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{16}ClNO.C_4H_4O_4$, calculated on the dried basis.

Category. Antipsychotic.

Description. A white to off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *asenapine maleate IPRS* or with the reference spectrum of asenapine maleate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *acetonitrile* and 40 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *asenapine maleate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.1 per cent v/v solution of *triethylamine* in *water*, adjusted to pH 2.5 with *perchloric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
5	70	30
20	20	80
30	20	80
32	70	30
40	70	30

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Maleic acid. 28.0 to 32.0 per cent.

Dissolve 0.1 g in 50.0 ml of *methanol*. Titrate with *0.1 M sodium hydroxide*, determining the end point Potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.011607 g $C_4H_4O_4$.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°, for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *acetonitrile* and 40 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 10.0 ml of the solution to 50.0 ml with solvent mixture.

Reference solution. A 0.02 per cent w/v solution of *asenapine maleate IPRS* in the solvent mixture.

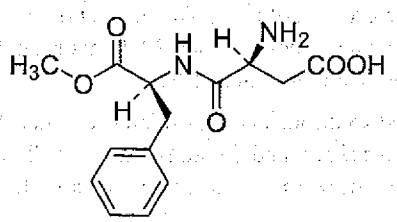
Chromatographic system
- a stainless steel column 25cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of 0.1 per cent v/v solution of *triethylamine* in *water*, adjusted to pH 2.5 with *perchloric acid* and 40 volumes of *acetonitrile*,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{16}ClNO.C_4H_4O_4$.

Aspartame



$C_{14}H_{18}N_2O_5$ Mol. Wt. 294.3

Aspartame is *N*-L-α-aspartyl-L-phenylalanine-1-methyl ester.

Aspartame contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{18}N_2O_5$, calculated on the dried basis.

Category. Pharmaceutical aid (sweetening agent).

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aspartame IPRS*.

B. When examined in the range 230 nm to 300 nm (2.4.7), a 0.1 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 247 nm, 252 nm, 258 nm and 264 nm.

Tests

pH (2.4.24). 4.0 to 6.0, determine in a 0.8 per cent w/v solution.

Specific optical rotation (2.4.22). +14.5° to +16.5°, determined at 20° in a 4.0 per cent w/v solution in *15 M formic acid* within 30 minutes of preparing the solution.

Light absorption (2.4.7). Absorbance of a 1.0 per cent w/v solution in *2 M hydrochloric acid*, prepared with the aid of ultrasound, at the maximum at about 430 nm, not more than 0.022.

5-Benzyl-3,6-dioxo-2-piperazineacetic acid. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of a mixture of 10 volumes of *methanol* and 90 volumes of *water*.

Reference solution. A 0.0075 per cent w/v solution of *5-benzyl-3,6-dioxo-2-piperazine-acetic acid IPRS* in a mixture of 10 volumes of *methanol* and 90 volumes of *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (3 to 10 µm),
- mobile phase: dissolve 5.6 g of *potassium dihydrogen phosphate* in 820 ml of *water*, adjusted to pH 4.3 with *phosphoric acid* and dilute to 1000 ml with *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviations for replicate injections is not more than 4.0 per cent and the tailing factor of the principal peak is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the response obtained for any peak at a retention time corresponding to that of *5-benzyl-3,6-dioxo-2-piperazineacetic acid IPRS* is not greater than the response obtained for the peak in the chromatogram of the reference solution corresponding to not more than 1.5 per cent of 5-benzyl-3,6-dioxo-2-piperazineacetic acid.

Other Related substances. Carry out the test for 5-Benzyl-3,6-dioxo-2-piperazineacetic acid, using reference solution (b) prepared by diluting 2.0 ml of the test solution to 100 ml with a mixture of 10 volumes of *methanol* and 90 volumes of *water*.

Inject 20 µl of reference solution (b) and the test solution, record the chromatograms and measure the peak responses. Continue elution of the test solution for twice the retention time of the aspartame peak. The sum of the areas of any peaks observed in the chromatogram obtained with the test solution, other than the peaks for aspartame and 5-benzyl-3,6-dioxo-2-piperazineacetic acid, is not more than the area of the aspartame peak obtained with reference solution (b) (2.0 per cent).

Arsenic (2.3.10). Mix 3.3 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, dissolve the cooled residue in 16 ml of *brominated hydrochloric acid AsT* and add 45 ml of *water*. Remove the excess of *bromine* with 2 ml of *stannous chloride AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on 2.0 g.

Loss on drying (2.4.19). Not more than 4.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

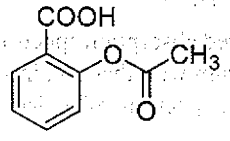
Assay. Dissolve 0.3 g in 1.5 ml of *anhydrous formic acid*, add 60 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration. A blank titration of more than 0.1 ml may be indicative of excessive water content. In such a case, repeat the test after taking precautions to maintain anhydrous conditions throughout.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02943 g of $C_{14}H_{18}N_2O_5$.

Storage. Store protected from light and moisture.

Aspirin

Acetylsalicylic Acid



$C_9H_8O_4$ Mol. Wt. 180.2

Aspirin is 2-acetoxybenzoic acid.

Aspirin contains not less than 99.5 per cent and not more than 100.5 per cent of $C_9H_8O_4$, calculated on the dried basis.

Category. Non-steroidal antiinflammatory; antirheumatic; antithrombotic.

Description. Colourless crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6) Compare the spectrum with that obtained with *aspirin IPRS* or with the reference spectrum of aspirin.

B. Boil about 0.5 g with 10 ml of *sodium hydroxide solution* for 3 minutes, cool and add 10 ml of *dilute sulphuric acid*; a white, crystalline precipitate is produced and the odour of acetic acid is perceptible. Filter, dissolve the precipitate in about 2 ml of *water* and add *ferric chloride test solution*; a deep violet colour is produced.

C. To the filtrate obtained in test B add 3 ml of *ethanol (95 per cent)* and 3 ml of *sulphuric acid* and warm; the odour of ethyl acetate is perceptible.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

Clarity of solution in alkali. A 5.0 per cent w/v solution in a warm 5 per cent w/v solution of *sodium carbonate* is clear (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 0.1 g of the substance under examination in *acetonitrile* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 50 mg of *salicylic acid IPRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *salicylic acid IPRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. To 1.0 ml of the solution, add 0.2 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.2 volume of *orthophosphoric acid*, 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the 2 principal peaks is not less than 6.0.

The relative retention time with reference to acetylsalicylic acid for 4-hydroxybenzoic acid (aspirin impurity A) is about 0.7; for 4-hydroxyisophthalic acid (aspirin impurity B) is about 0.8; for salicylic acid (aspirin impurity C) is about 1.3; for acetylsalicylsalicylic acid (aspirin impurity D) is about 2.3; for salicylsalicylic acid (aspirin impurity E) is about 3.2; for acetylsalicylic anhydride (aspirin impurity F) is about 6.0.

Inject reference solution (a) and the test solution. Run the chromatogram 7 times the retention time of the acetylsalicylic acid peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to aspirin impurities A, B, C, D, E and F is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent). The sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent). Ignore any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Arsenic (2.3.10). Mix 5.0 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Chlorides (2.3.12). Boil 1.75 g with 75 ml of *water* for 5 minutes, cool, add sufficient *water* to restore the original volume and filter. 25 ml of the filtrate complies with the limit test for chlorides (430 ppm).

Sulphates (2.3.17). 10 ml of the filtrate obtained in the test for Chlorides complies with the limit test for sulphates (650 ppm).

Readily carbonisable substances. Dissolve 0.5 g in 5 ml of *sulphuric acid* (containing 94.5 per cent to 95.5 per cent w/w of H_2SO_4); any colour produced is not more intense than that of reference solution BYS4 (2.4.1).

Heavy metals. Not more than 10 ppm, determined by the following method. Dissolve 2.0 g in 25 ml of *acetone*, add 1 ml of *water* and 10 ml of *hydrogen sulphide solution*; any colour produced is not more intense than that produced by mixing 25 ml of *acetone*, 1.0 ml of *lead standard solution (20 ppm Pb)* and 10 ml of *hydrogen sulphide solution*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 25.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.05 per cent w/v solution of *aspirin IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 600 volumes of *water* and 400 volumes of *acetonitrile*, add 2 ml of *orthophosphoric acid*, filter,
- flow rate: 1 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 10 µl.

The retention time of the principal peak is about 4.0 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent

Inject the reference solution and the test solution.

Calculate the content of $C_9H_8O_4$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Aspirin Tablets

Acetylsalicylic Acid Tablets

Aspirin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aspirin, $C_9H_8O_4$.

Usual strengths. 75 mg; 150 mg; 300 mg; 600 mg.

Identification

Disperse a quantity of powdered tablets containing 0.5 g Aspirin with 20.0 ml of *ethanol,* Filter. Evaporate the filtrate and dry the residue at 60° for 1 hour. The residues comply with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aspirin IPRS* or with the reference spectrum of aspirin.

Tests

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium: 500 ml of a buffer solution pH 4.5 prepared by dissolving 2.99 g of *sodium acetate* and 1.66 ml of *glacial acetic acid* with sufficient *water* and dilute to 1000 ml with *water,*

Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute a suitable volume of the filtrate with the dissolution medium and measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7). Calculate the content of $C_9H_8O_4$ in the medium from the absorbance obtained from a solution of known concentration of *aspirin IPRS* in the same medium.

Q. Not less than 70 per cent of the stated amount of $C_9H_8O_4$

Related substances. Determine by liquid chromatography (2.4.14),

NOTE — Prepare the solutions immediately before use.

Test solution. Disperse a quantity of the powdered tablets containing 0.1 g of Aspirin in 40 ml of *acetonitrile* with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A 0.003 per cent w/v solution of *salicylic acid (aspirin impurity C)* in the mobile phase.

Reference solution (c). Dilute 5.0 ml of reference solution (b) and 1.0 ml of the test solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile*, 60 volumes of *water* and 0.2 volume of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 20 µl.

Name	Relative retention time
Aspirin impurity A[1]	0.6
Aspirin impurity B[2]	0.7
Aspirin (Retention time: about 5 minutes)	1.0
Aspirin impurity C[3]	1.4
Aspirin impurity F[4]	8.0

[1]4-hydroxybenzoic acid,

[2]4-hydroxyisophthalic acid,

[3]salicylic acid,

[4]acetylsalicylic anhydride.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to aspirin and aspirin impurity C is not less than 6.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 1.2 times the retention time of aspirin impurity F. In the chromatogram obtained with the test solution, the area of any peak corresponding to aspirin impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all other secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), using chromatographic system and reference solution (c) as described under Related substances.

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 60 mg of Aspirin in 40 ml of *acetonitrile,* with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with *water*.

Reference solution. A 0.06 per cent w/v solution of *aspirin IPRS* in the mobile phase.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to aspirin and aspirin impurity C is not less than 6.0.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_8O_4$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states, if the tablets are dispersible should be dispersed in water immediately before use.

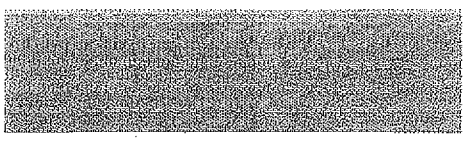
Soluble Aspirin Tablets

Soluble Acetylsalicylic Acid Tablets; Effervescent Soluble Aspirin Tablets; Effervescent Aspirin Tablets; Calcium Aspirin Tablets.

Soluble Aspirin Tablets contain Aspirin in a suitable soluble, effervescent base.

Soluble Aspirin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aspirin, $C_9H_8O_4$.

Usual strength. 300 mg.



Identification

A. Disperse a quantity of powdered tablets containing 0.5 g Aspirin with 20.0 ml of *ethanol* and filter.Evaporate the filtrate and dry the residue at 60° for 1 hour. The residues comply with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *aspirin IPRS* or with the reference spectrum of aspirin.

B. Dissolve with vigorous effervescence on the addition of warm *water* to produce a clear solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14),

NOTE — Prepare the solutions immediately before use.

Test solution. Disperse a quantity of the powdered tablets containing 0.1 g of Aspirin in 40 ml of *acetonitrile* with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A 0.003 per cent w/v solution of *salicylic acid (aspirin impurity C)* in the mobile phase.

Reference solution (c). Dilute 5.0 ml of reference solution (b) and 1.0 ml of the test solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile*, 60 volumes of *water* and 0.2 volume of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 20 µl.

Name	Relative retention time
Aspirin impurity A[1]	0.6
Aspirin impurity B[2]	0.7
Aspirin (Retention time: about 5 minutes)	1.0
Aspirin impurity C[3]	1.4
Aspirin impurity F[4]	8.0

[1]4-hydroxybenzoic acid,

[2]4-hydroxyisophthalic acid,

[3]salicylic acid,

[4]acetylsalicylic anhydride.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to aspirin and aspirin impurity C is not less than 6.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 1.2 times the retention time of aspirin impurity F. In the chromatogram obtained with the test solution, the area of any peak corresponding to aspirin impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all other secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), using chromatographic system and reference solution (c) as described under Related substances.

NOTE —Prepare the solutions immediately before use.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 60 mg of Aspirin in 40 ml of *acetonitrile,* with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with *water*.

Reference solution. A 0.06 per cent w/v solution of *aspirin IPRS* in the mobile phase.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to aspirin and aspirin impurity C is not less than 6.0.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_8O_4$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Aspirin Gastro-resistant Tablets

Acetylsalicylic Acid Gastro-resistant Tablets

Aspirin Gastro-resistant Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of aspirin, $C_9H_8O_4$.

Usual strength. 75 mg; 150 mg.

Identification

Boil a quantity of the powdered tablets containing 0.3 g of Aspirin for 2 to 3 minutes with 10 ml of *5 M sodium hydroxide*, cool and add an excess of *1 M sulphuric acid*;a crystalline

precipitate is produced. To a solution of the precipitate in *water* add *iron(III) chloride solution;* a deep violet colour is produced.

Tests

Dissolution (2.5.2).

A. Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 120 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution immediately, suitably diluted with the dissolution medium, if necessary, at the maximum at about 276 nm (2.4.7). Calculate the content of $C_9H_8O_4$ in the medium from the absorbance obtained from a solution of known concentration of *aspirin IPRS,* prepared by dissolving in *0.1 M hydrochloric acid.*

Complies with the acceptance criteria given under acid stage.

B. Apparatus No. 1 (Basket),

Medium. 900 ml of *0.1 M hydrochloric acid* replace with *mixed phosphate buffer pH 6.8,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution immediately, suitably diluted with the dissolution medium, if necessary, at the maximum at about 265 nm (2.4.7). Calculate the content of $C_9H_8O_4$ in the medium from the absorbance obtained from a solution of known concentration of *aspirin IPRS,* prepared by dissolving in the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_9H_8O_4$.

Salicylic acid. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 0.3 g of Aspirin in 60 ml of *acetonitrile* and 1 ml of *formic acid* for 15 minutes and dilute to 100.0 ml with *acetonitrile*, filter.

Reference solution (a). A 0.009 per cent w/v solution of *salicylic acid IPRS* in a mixture of 99 volumes of *acetonitrile* and 1 volume of *formic acid.*

Reference solution (b). A solution containing 0.3 per cent w/v of *aspirin IPRS* and 0.009 per cent w/v of *salicylic acid IPRS* in a mixture of 99 volumes of *acetonitrile* and 1 volume of *formic acid.*

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of *0.05 M sodium dihydrogen orthophosphate*, adjusted to pH 2.0 with *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to salicylic acid is not more than the area of the peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

Other tests. Comply with the tests under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 99 volumes of *acetonitrile* and 1 volume of *formic acid.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.3 g of Aspirin with 60 ml of *acetonitrile* and 1 ml of *formic* for 15 minutes and dilute to 100.0 ml with *acetonitrile,* filter. Dilute 1.0 ml of the solution to 4.0 ml with the solvent mixture.

Reference solution (a). A 0.075 per cent w/v solution of *aspirin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.075 per cent w/v of *aspirin IPRS* and 0.0015 per cent w/v of *salicylic acid* in the solvent mixture.

Use chromatographic system as described in test for Salicylic acid.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_9H_8O_4$ in the tablets.

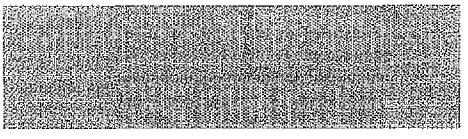
Storage. Store protected from moisture.

Labelling. The label states that the tablets should be swallowed whole and not chewed.

Aspirin Gastro-resistant and Atorvastatin Capsules

Aspirin Gastro-resistant and Atorvastatin Calcium Capsules; Acetylsalicylic Acid Gastro-resistant and Atorvastatin Calcium Capsules

Aspirin Gastro resistant and Atorvastatin Capsules contain atorvastatin calcium equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atorvastatin, $C_{33}H_{35}FN_2O_5$ and aspirin, $C_9H_8O_4$.



Usual strengths. Aspirin, 75 mg and Atorvastatin, 10 mg; Aspirin, 150 mg and Atorvastatin, 10 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

For Aspirin —

A. Apparatus No. 1 (Basket),
Medium. 1000 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 120 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 15 mg of *aspirin IPRS* in 40 ml of *methanol* and dilute to 100.0 ml with the dissolution medium. Dilute 5.0 ml of the solution to 100.0 ml with the dissolution medium.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- sample temperature: 15°.
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 7.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and adjusted to pH 2.0 with *orthophosphoric acid* and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_8O_4$ in the medium.

Complies with the acceptance criteria given under acid stage.

B. Apparatus No. 1 (Basket),
Medium. 1000 ml of *mixed phosphate buffer pH 6.8*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 15 mg of *aspirin IPRS* in 40 ml of *methanol* and dilute to 100.0 ml with the dissolution medium. Dilute 5.0 ml of the solution to 100.0 ml with the dissolution medium.

Use chromatographic system as described in stage A.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_8O_4$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_9H_8O_4$.

For Atorvastatin —

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *atorvastatin calcium IPRS* in *methanol*, and dilute with the dissolution medium to obtain a solution having a known concentration similar to the test solution.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature 15°,
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 1.54 g of *ammonium acetate* in 1000 ml of *water* and adjusted to pH 4.0 with *glacial acetic acid* and 50 volumes of a mixture of 92.5 volumes of *acetonitrile* and 7.5 volumes of *tetrahydrofuran*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 246 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{33}H_{35}FN_2O_5$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{33}H_{35}FN_2O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

For Aspirin —

Use the solvent mixture and chromatographic system as described under Assay.

Test solution. Disperse a quantity of the mixed content containing 300 mg of Aspirin with 60 ml of the solvent mixture, with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the solvent mixture, filter.

Reference solution (a). A 0.009 per cent w/v solution of *salicylic acid* in the solvent mixture.

Reference solution (b). A solution containing 0.009 per cent w/v of *salicylic acid* and 0.3 per cent w/v of *aspirin IPRS* in the solvent mixture.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to salicylic acid and aspirin is not less than 3.0 in the chromatogram obtained with reference solution (b), the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to salicylic acid is not more than the area of the peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

For Atorvastatin —

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water.*

Solution A. Dissolve 5.75 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water.*

Solution B. 92.5 volumes of *acetonitrile* and 7.5 volumes of *tetrahydrofuran.*

NOTE — Prepare the solutions immediately before use.

Test solution. Disperse a quantity of the mixed content containing 50 mg of Atorvastatin with 10 ml of *methanol*, add 20 ml of the solvent mixture, with the aid of ultrasound for 20 minutes and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). Dissolve 55 mg of *atorvastatin calcium IPRS* in 5 ml of *methanol* and dilute to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 15°,
- mobile phase: A. a mixture of 58 volumes of solution A and 42 volumes of solution B;
  B. a mixture of 20 volumes of solution A, 20 volumes of solution B and 60 volumes of *methanol*,
- a gradient programme using the conditions given below,
- spectrophotometer set at 246 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Flow rate (ml/minute)
0	100	0	1.8
20	100	0	1.8
35	25	75	1.5
40	25	75	1.5
55	0	100	1.5
60	100	0	1.8
70	100	0	1.8

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent). Ignore any peak with an area less than 0.05 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent). Ignore peaks due to aspirin and salicylic acid.

Uniformity of content. Complies with the test stated under Capsules.

For Atorvastatin —

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse content of one capsule in 20 ml of *methanol*, with the aid of ultrasound for 30 minutes and dilute to obtain a solution having the similar concentration of the reference solution with the solvent mixture.

Calculate the content of $C_{33}H_{35}FN_2O_5$ in the capsule.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of mobile phase A and 40 volumes of *acetonitrile.*

Test solution. Weigh and mix the content of 20 capsules. Disperse a quantity of mixed content containing 75 mg of Aspirin in 20 ml of *methanol*, with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with the solvent mixture and filter. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.15 per cent w/v solution of *aspirin IPRS* in the solvent mixture.

Reference solution (b). A 0.055 per cent w/v solution of *atorvastatin calcium IPRS* in *methanol.*

Reference solution (c). Dilute a suitable volume of reference solution (a) and reference solution (b) with the solvent mixture to obtain a solution having a concentration similar to the test solution.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 15°,

- mobile phase: A. a buffer solution prepared by dissolving 1.4 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
15	70	30
17	40	60
30	40	60
32	70	30
35	70	30

The elution order of peaks is aspirin and atorvastatin.

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for both the peaks.

Inject reference solution (c) and the test solution.

Calculate the content of $C_9H_8O_4$ and $C_{33}H_{35}FN_2O_5$ in the capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the quantity of atorvastatin calcium in the terms of the equivalent amount of atorvastatin and aspirin.

Aspirin Gastro-resistant and Rosuvastatin Capsules

Aspirin Gastro-resistant and Rosuvastatin Calcium Capsules; Acetylsalicylic Acid Gastro-resistant and Rosuvastatin Calcium Capsules

Aspirin Gastro resistant and Rosuvastatin Capsules contain rosuvastatin calcium equivalent to not less than 90.0 per cent and not more than 110.0 percent of the stated amount of rosuvastatin, $C_{22}H_{28}FN_3O_6S$ and aspirin, $C_9H_8O_4$.

Usual strengths. Aspirin, 75 mg and Rosuvastatin, 5 mg; Aspirin, 75 mg and Rosuvastatin, 10 mg; Aspirin, 150 mg and Rosuvastatin, 10 mg; Aspirin, 75 mg and Rosuvastatin, 20 mg; Aspirin, 150 mg and Rosuvastatin, 20 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

For Aspirin —

A. Apparatus No. 1 (Basket),
Medium. 1000 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 120 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 15 mg of *aspirin IPRS* in 5 ml of *acetonitrile* and dilute with the dissolution medium to obtain a solution having a concentration similar to the test solution.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS 2),
- sample temperature: 15°.
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 1.74 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.3 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 10 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_8O_4$ in the medium.

Complies with the acceptance criteria given under acid stage.

B. Apparatus No. 1 (Basket),
Medium. 1000 ml of a buffer solution prepared by mixing 75 volumes of *0.1 M hydrochloric acid* and 25 volumes of *0.2 M trisodium phosphate dodecahydrate*, adjusted to pH 6.8 with *2 M hydrochloric acid* or *2 M sodium hydroxide*,
Speed and time. 100 rpm and 90 minutes.

Withdraw a suitable volume of the medium and filter.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 15 mg of *aspirin IPRS* in 5 ml of *acetonitrile* and dilute with the dissolution medium to obtain a solution having a concentration similar to the test solution.

Use chromatographic system and system suitability as described under stage A.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_8O_4$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_9H_8O_4$.

For Rosuvastatin —

Apparatus No. 2 (Paddle),

Medium. 900 ml of phosphate buffer pH 6.8 prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 6.8 with *sodium hydroxide solution*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.055 per cent w/v solution of *rosuvastatin calcium IPRS* in equal volumes of *acetonitrile* and *water*. Dilute the solution with the dissolution medium to obtain a solution having a concentration similar to the test solution.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 1.74 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and adjusted to pH 2.2 with *orthophosphoric acid*, 42.5 volumes of *acetonitrile* and 7.5 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{28}FN_3O_6S$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{22}H_{28}FN_3O_6S$.

Related substances. Determine by liquid chromatography (2.4.14).

For Aspirin —

Solvent mixture. 90 volumes of *acetonitrile* and 10 volumes of *water*.

Test solution. Disperse a quantity of the mixed powdered content of capsules containing 375 mg of Aspirin in 75 ml of the solvent mixture, with the aid of ultrasound with intermittent shaking and dilute to 100.0 ml with the solvent mixture. Dilute a suitable volume of the solution with the solvent mixture to obtain a concentration of 0.075 per cent w/v of Aspirin.

Reference solution. A 0.00075 per cent w/v solution of *aspirin IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS 3V),
- sample temperature: 15°,
- mobile phase: A. *0.01 M potassium dihydrogen orthophosphate*, adjusted to pH 2.5 with *orthophosphoric acid*,

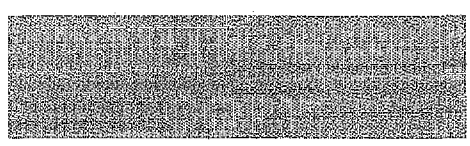
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
15	60	40
25	20	80
26	60	40
35	60	40

Name	Relative retention time	Correction factor
Aspirin	1.0	---
Salicylic acid	1.48	0.86

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to salicylic acid is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (3.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak obtain with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks except salicylic acid is not more than twice the area of the principal peak in the



chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent). Ignore any peak due to rosuvastatin.

For Rosuvastatin —

Test solution. Disperse a quantity of the mixed powdered content of capsules containing 25 mg of Rosuvastatin in 30 ml of the mobile phase, with the aid of ultrasound for 30 minutes with intermittent shaking and dilute to 50.0 ml with the mobile phase.

Reference solution. A 0.00055 per cent w/v solution of *rosuvastatin calcium IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 59 volumes of a buffer solution prepared by dissolving 1.54 g of *ammonium acetate* in 900 ml of *water*, adjusted to pH 4.0 with *glacial acetic acid* and dilute to 1000.0 ml with *water*, 36 volumes of *acetonitrile* and 5 volumes of *tetrahydrofuran*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. Run the chromatogram 6 times the retention time of the rosuvastatin peak. The area of any secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of principal peak in the chromatogram obtained with the reference solution (3.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent). Ignore any peak due to salicylic acid and aspirin.

Uniformity of content. Complies with the test stated under Capsules.

For Rosuvastatin —

Determine by liquid chromatography (2.4.14), as described under Assay for Rosuvastatin with the following modifications.

Test solution. Disperse content of one capsule in 60 ml of the mobile phase, with the aid of ultrasound for 30 minutes and dilute to obtain a solution having the similar concentration of the reference solution with the mobile phase.

Calculate the content of $C_{22}H_{28}FN_3O_6S$ in the capsule.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

For Aspirin —

Solvent mixture. 90 volumes of *acetonitrile* and 10 volumes of *water*.

Test solution. Weigh and mix the content of 20 capsules. Disperse a quantity of powder containing 375 mg of Aspirin in 75 ml of the solvent mixture, with the aid of ultrasound with intermittent shaking and dilute to 100.0 ml with the solvent mixture. Dilute a suitable volume of the solution with the solvent mixture to obtain a concentration of 0.075 per cent w/v of Aspirin.

Reference solution. A 0.075 per cent w/v solution of *aspirin IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 15°,
- mobile phase: a mixture of 60 volumes of *0.01M potassium dihydrogen orthophosphate*, adjusted to pH 2.5 with *orthophosphoric acid*, and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_8O_4$ in the capsules.

For Rosuvastatin —

Test solution. Disperse a quantity of the mixed powered content of capsules containing 50 mg of Rosuvastatin in 200 ml of the mobile phase, with the aid of ultrasound for 30 minutes with intermittent shaking and dilute to 250.0 ml with the mobile phase. Dilute a suitable volume of the solution with the mobile phase to obtain a concentration of 0.005 per cent w/v of Rosuvastatin.

Reference solution. A 0.0055 per cent w/v solution of *rosuvastatin calcium IPRS* in the mobile phase.

Use chromatographic system as described under Related substances of Rosuvastatin.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the

tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{28}FN_3O_6S$ in the capsules.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the quantity of rosuvastatin calcium in term of the equivalent amount of rosuvastatin and aspirin.

Aspirin and Caffeine Tablets

Acetylsalicylic Acid and Caffeine Tablets

Aspirin and Caffeine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of aspirin, $C_9H_8O_4$ and caffeine, $C_8H_{10}N_4O_2$.

Usual strength. Aspirin 0.4 g and caffeine 30 mg.

Identification

A. Boil 1 g of the powdered tablets with 10 ml of *1 M sodium hydroxide*, cool and filter. Acidify the filtrate with *1 M sulphuric acid*; a white precipitate is produced. Dissolve the precipitate in about 2 ml of *water* and add *ferric chloride test solution*; a deep violet colour is produced.

B. Shake 0.5 g of the powdered tablets with 10 ml of *water* for 5 minutes, filter and add 10 ml of *1 M sodium hydroxide*. Extract with three quantities, each of 30 ml of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the combined extracts through absorbent cotton and evaporate the filtrate to dryness. Reserve a quantity of the residue for test C. Dissolve 10 mg of the residue in 1 ml of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness in a porcelain dish; a reddish residue remains which becomes purple on exposure to ammonia vapour.

C. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001per cent w/v solution of the residue reserved in Test B shows an absorption maximum at about 273 nm.

Tests

Dissolution (2.5.2).

For aspirin —

Apparatus No. 2 (Paddle),
Medium. 500 ml of a pH 4.5 buffer prepared by dissolving 2.99 g of *sodium acetate* and 1.66 ml of *glacial acetic acid* in sufficient *water* and dilute to 1000 ml with *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the dissolution medium, if necessary, at the maximum at about 265 nm (2.4.7). Calculate the content of $C_9H_8O_4$ in the medium from the absorbance obtained from a solution of known concentration of *aspirin IPRS* in the dissolution medium in such manner to get similar concentration of the test solution.

Q. Not less than 75 per cent of the stated amount of $C_9H_8O_4$.

Salicylic acid. Disperse a quantity of the powdered tablets containing 0.5 g of Aspirin with 50.0 ml of *chloroform* and 10 ml of *water* and allow to separate. Filter the chloroform layer through a dry filter paper and evaporate 10 ml of the filtrate to dryness at room temperature using a rotary evaporator. To the residue add 4 ml of *ethanol (95 per cent)*, stir well, dilute to 100 ml with *water* at a temperature not exceeding 10°, filter immediately, rapidly transfer 50 ml to a Nessler cylinder, add 1 ml of freshly prepared *acid ferric ammonium sulphate solution*, mix and allow to stand for 1 minute; the violet colour produced is not more intense than that produced by adding 1 ml of *acid ferric ammonium sulphate solution* to a mixture of 3.0 ml of a freshly prepared 0.05 per cent w/v solution of *salicylic acid*, 2 ml of *ethanol (95 per cent)* and sufficient *water* to produce 50 ml contained in a second Nessler cylinder (3.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets.

For aspirin — Weigh a quantity of the powder containing about 0.7 g of Aspirin, add 20 ml of *water* and 2 g of *sodium citrate* and heat under a reflux condenser for 30 minutes. Cool, wash the condenser with 30 ml of warm *water* and titrate with *0.5 M sodium hydroxide* using *phenolphthalein solution* as indicator.

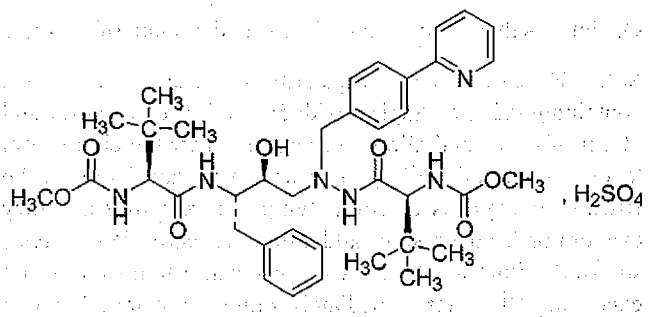
1 ml of *0.5 M sodium hydroxide* is equivalent to 0.04504 g of $C_9H_8O_4$.

For caffeine — Weigh a quantity of the powder containing about 30 mg of Caffeine add 200 ml of *water* and shake for 30 minutes. Add sufficient *water* to produce 250.0 ml and filter. To 10.0 ml of the filtrate add 10 ml of *1 M sodium hydroxide* and extract immediately with five quantities, each of 30 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the combined chloroform extracts, if necessary, through absorbent cotton previously moistened with *chloroform*. Evaporate the solution to dryness and dissolve the residue as completely as possible in *water*, warming gently if necessary. Cool, add sufficient *water* to produce 100.0 ml, mix and filter if necessary. Measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7).

Calculate the content of $C_8H_{10}N_4O_2$ taking 504 as the specific absorbance at 273 nm.

Storage. Store protected from moisture.

Atazanavir Sulphate



$C_{38}H_{52}N_6O_7.H_2SO_4$ Mol. Wt. 802.9

Atazanavir Sulphate is salt with sulphuric acid of (3*S*,8*S*,9S,12*S*)-3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl]methyl]-2,5,6,10,13-pentaazatetradecanedioic acid dimethyl ester.

Atazanavir Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{38}H_{52}N_6O_7.H_2SO_4$, calculated on the dried basis.

Category. Antiretroviral.

Description. A white to pale yellow crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atazanavir sulphate IPRS* or with the reference spectrum of atazanavir sulphate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). – 48° to – 44°, determined on 1.0 per cent w/v solution in a mixture of equal volumes of *methanol* and *water* at 22°.

Sulphuric acid. Not less than 11.0 per cent w/w and not more than 13.0 per cent w/w.

Weigh 0.2 g and sonicate with 30 ml of *methanol*, add 30 ml of *water*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0049 g of sulphuric acid.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Dissolve 0.1 g of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution. A 0.0005 per cent w/v solution of *atazanavir sulphate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3),
- column temperature: 45°,
- mobile phase: A. dissolve 6.0 g of *sodium dihydrogen orthophosphate monohydrate* in *water*, add 2.0 ml of *orthophosphoric acid* and dilute to 1000 ml with *water*, adjusted to pH 2.5 with *triethylamine*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
15	60	40
32	30	70
44	30	70
45	60	40
55	60	40

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *atazanavir sulphate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (Such as Inertsil ODS-3),
- column temperature: 45°,
- mobile phase. a mixture of 45 volumes of buffer solution prepared by dissolving 6.0 g of *sodium dihydrogen orthophosphate monohydrate* in *water*, add 2.0 ml of *orthophosphoric acid* and dilute to 1000 ml with *water*, adjusted to pH 2.5 with *triethylamine* and 55 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency are not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{52}N_6O_7.H_2SO_4$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Atazanavir Capsules

Atazanavir Sulphate Capsules

Atazanavir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atazanavir, $C_{38}H_{52}N_6O_7$.

Usual strengths. 150 mg; 200 mg; 300 mg; 400 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with that of reference solution.

B. When examined in the range from 200 nm to 400 nm (2.4.7), a 0.012 per cent w/v solution in 0.2 per cent v/v solution of *hydrochloric acid* shows an absorption maximum as obtained with *atazanavir sulphate IPRS* of the same concentration.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle) (Use sinkers, if required),
Medium. 1000 ml of *0.025 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary, with the dissolution medium. Measure the absorbance at the maximum at about 300 nm (2.4.7). Calculate the content of $C_{38}H_{52}N_6O_7$ in the medium from the absorbance obtained from a solution of known concentration of *atazanavir sulphate IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{38}H_{52}N_6O_7$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 10 volumes of mobile phase A and 10 volumes of mobile phase B.

Test solution. Mix the contents of 20 capsules. Disperse the content of capsules containing about 50 mg of Atazanavir with 30 ml of the solvent mixture, sonicate for 15 minutes and dilute to 50.0 ml with the solvent mixture and filter.

Reference solution. A solution of *atazanavir sulphate IPRS* containing 0.001 per cent w/v of atazanavir in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3),
- column temperature: 45°,
- mobile phase: A. dissolve 6.0 g of *sodium dihydrogen orthophosphate monohydrate* and 2 ml of *orthophosporic acid* in 1000 ml of *water*, adjusted to pH 2.5 with *triethylamine or orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume:20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
15	60	40
32	30	70
44	30	70
45	60	40
55	60	40

Inject the reference solution. The test is not valid unless the theoretical plates of the principal peak is not less than 2000 and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any

secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and mix the contents of 20 capsules. Disperse a quantity of the mixed content containing about 20 mg of Atazanavir with 150 ml of the mobile phase, sonicate for 15 minutes and dilute to 250.0 ml with the mobile phase, filter.

Reference solution. A 0.009 per cent w/v solution of *atazanavir sulphate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS),
- column temperature: 40°,
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{52}N_6O_7$ in the capsules.

Storage. Store at a temperature not exceeding 30°.

Atazanavir and Ritonavir Tablets

Atazanavir Sulphate and Ritonavir Tablets

Atazanavir and Ritonavir Tablets contain atazanavir sulphate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atazanavir, $C_{38}H_{52}N_6O_7$ and ritonavir, $C_{37}H_{48}N_6O_5S_2$.

Usual strength. Atazanavir, 300 mg and Ritonavir, 100 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of a solution prepared by dissolving 37.7 g of *polyoxyethylene 10 lauryl ether* in 1000 ml of *water* and add 2.1 ml of *hydrochloric acid*,

Speed and time. 100 rpm and 45 minutes for atazanavir and 120 minutes for ritonavir.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14),

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 95 mg of *atazanavir sulphate IPRS* and 28 mg of *ritonavir IPRS* in 10 ml of *methanol*, with the aid of ultrasound and dilute to 50.0 ml with the dissolution medium. Further, dilute 10.0 ml of the solution to 50.0 ml with the dissolution medium.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 49 volumes of a buffer solution prepared by dissolving 3.4 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 51 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3500 theoretical plates for atazanavir, the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent for both peaks.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{52}N_6O_7$ and $C_{37}H_{48}N_6O_5S_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{38}H_{52}N_6O_7$ and 80 per cent of the stated amount of $C_{37}H_{48}N_6O_5S_2$.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse a quantity of the powdered tablets containing 300 mg of Atazanavir in 140 ml of the mobile phase with the aid of ultrasound for 30 minutes with intermittent shaking, dilute to 200.0 ml with the mobile phase and filter.

Inject the test solution. The area of any secondary peak is not more than 2.5 per cent and the sum of areas of all the secondary peaks is not more than 5.0 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 300 mg of atazanavir in 140 ml of the mobile phase with the aid of ultrasound and intermittent shaking and dilute to 200.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (a). A 0.085 per cent w/v of *atazanavir sulphate IPRS* in the mobile phase.

Reference solution (b). A 0.025 per cent w/v of *ritonavir IPRS* in the mobile phase.

Reference solution (c). Dilute 5.0 ml each of reference solution (a) and (b) to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Zorbax Eclips XDB C-8),
- mobile phase: a mixture of 55 volumes of *water* and 45 volumes of *acetonitrile,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Inject reference solution (c).The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for each components.

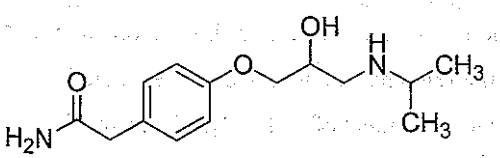
Inject reference solution (c) and the test solution.

Calculate the contents of $C_{38}H_{52}N_6O_7$ and $C_{37}H_{48}N_6O_5S_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the quantity of atazanavir sulphate in the terms of the equivalent amount of atazanavir and ritonavir.

Atenolol



$C_{14}H_{22}N_2O_3$ Mol. Wt. 266.3

Atenolol is (*RS*)-4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide.

Atenolol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{22}N_2O_3$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atenolol IPRS* or with the reference spectrum of atenolol.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *methanol* shows absorption maxima at about 275 nm and 282 nm. The ratio of the absorbance at the maximum at about 275 nm to that at the maximum at about 282 nm is 1.15 to 1.20.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 99 volumes of *methanol* and 1 volume of *strong ammonia solution.*

Test solution. Dissolve 1.0 g of the substance under examination in sufficient *methanol* to produce 100 ml.

Reference solution. A 1.0 per cent w/v solution of *atenolol IPRS* in *methanol.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 1.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: dissolve 1.1 g of *sodium 1-heptanesulphonate* and 0.71 g of *anhydrous dibasic sodium phosphate* in 700 ml of *water*. Add 2 ml of *dibutylamine*, and adjusted to pH 3.0 with *0.8 M orthophosphoric acid*, add 300 ml of *methanol*,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 226nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 6 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (0.25 per cent). The sum of areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (0.5 per cent).

Chlorides (2.3.12). Dissolve 0.25 g in a mixture of 1 ml of *2 M nitric acid* and 15 ml of *water*. The solution complies with the limit test for chlorides without further addition of *2 M nitric acid* (0.1 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.2 g in 80 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02663 g of $C_{14}H_{22}N_2O_3$.

Atenolol Tablets

Atenolol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of atenolol, $C_{14}H_{22}N_2O_3$.

Usual strengths. 50 mg; 100 mg.

Identification

A. Heat a quantity of the powdered tablets containing about 0.1 g of Atenolol with 15 ml of *methanol* to 50°, shake for 5 minutes, filter (Whatman No. 42 paper is suitable) and evaporate the filtrate to dryness on a water-bath. Warm the residue with 10 ml of *0.1 M hydrochloric acid*, shake and filter. Add to the filtrate sufficient *1 M sodium hydroxide* to make it alkaline, extract with 10 ml of *chloroform*, dry by shaking with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 105° for 1hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atenolol IPRS* or with the reference spectrum of atenolol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 275 nm and 282 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of acetate buffer pH 4.6 prepared by mixing 45 volumes of *0.1M sodium acetate* and 55 volumes of *0.1M acetic acid*, adjusted to pH 4.6 with *dilute sodium hydroxide* or *dilute acetic acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the mobile phase to obtain a solution containing 0.001 per cent w/v of Atenolol.

Reference solution. A 0.001 per cent w/v solution of *atenolol IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 1.57 g *sodium1-heptanesulphonate* and 1.0 g of *anhydrous dibasic sodium phosphate* and 2.85 ml of *dibutylamine* in 700ml of *water*, adjusted to pH 3.0 with *0.8 M phosphoric acid*, diluted to 1000 ml with *water* and 30 volumes of *methanol*,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{22}N_2O_3$ in the medium

Q. Not less than 80 per cent of the stated amount of $C_{14}H_{22}N_2O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 25 mg of Atenolol with 25 ml of the mobile phase and mix with the aid of ultrasound for 20 minutes, filter (Such as Whatman GF/C filter) and use the filtrate.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with the mobile phase.

Reference solution (b). Dissolve 10 mg of *atenolol impurity standard IPRS* in 0.1 ml of *dimethyl sulphoxide* with the aid of gentle heat, dilute to 10 ml with the mobile phase and mix.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: dissolve 0.8 g of *sodium octanesulphonate* and 0.4 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of a mixture of 20 volumes of *tetrahydrofuran*, 180 volumes of *methanol* and 800 volumes of a 0.34 per cent w/v solution of *potassium dihydrogen phosphate* and adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (b) resembles the reference chromatogram supplied with the atenolol impurity standard RS in that the peak due to bis-ether precedes and is separated from that due to tertiary amine, which is normally a doublet. If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase; if its concentration is increased, the retention time of the tertiary amine is prolonged.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 4-(2-hydroxy-3-isopropylamino-propoxy)phenylacetic acid (blocker acid) is not more than the area of the peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the area of any peak corresponding to either tertiary amine or bis-ether is not more than half the area of the peak in the chromatogram obtained with reference solution (a) (0.25 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.2 g of Atenolol, transfer to a 500-ml volumetric flask using 300 ml of *methanol*, heat the resulting suspension to 60° and shake for 15 minutes. Cool, dilute to 500.0 ml with *methanol*, filter through a fine glass micro-fibre filter paper (Whatman GF/C) and dilute a suitable volume of the filtrate with sufficient *methanol* to produce a solution containing 0.01 per cent w/v of Atenolol. Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of $C_{14}H_{22}N_2O_3$ taking 53.7 as the value of the specific absorbance at 275 nm.

Atenolol and Chlorthalidone Tablets

Atenolol and Chlorthalidone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of atenolol, $C_{14}H_{22}N_2O_3$ and not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorthalidone, $C_{14}H_{11}ClN_2O_4S$.

Usual Strengths. Atenolol, 25 mg and Chlorthalidone, 6.25 mg; Atenolol, 50 mg and Chlorthalidone, 12.5 mg; Atenolol, 100 mg and Chlorthalidone, 25 mg.

Identification

A. Determine by thin layer chromatography (2.4.17), using the plate coated with *silica gel GF254*.

Mobile phase. A mixture of 30 volumes of *18 M ammonia* and 150 volumes of *butan-1-ol*.

Test solution. Remove any film coating from the tablets. Disperse a quantity of the powdered tablets containing 0.1 g of Atenolol with 10.0 ml of *methanol* for 15 minutes and filter.

Reference solution (a). A 1.0 per cent w/v solution of *atenolol IPRS* in *methanol*.

Reference solution (b). A 0.25 per cent w/v solution of *chlorthalidone IPRS* in *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. After development, dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. The two principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and reference solution (b).

B. In the Assay, the principal peaks in the chromatogram obtained with test solution correspond the principal peaks in the chromatograms obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 100 volumes of *acetonitrile* and 3.2 volumes of 1.8 M *sulphuric acid*.

Test solution. Use the filtrate and, if necessary, dilute with the solvent mixture.

Reference solution. Dissolve a suitable quantity of *atenolol IPRS* and *chlorthalidone IPRS* in a mixture of 75 volumes of *water* and 22.5 volumes of the solvent mixture to obtain a solution having known concentration similar to the test solution.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 74 volumes of *water* and 25 volumes of *acetonitrile,* 0.8 volume of *1.8 M sulphuric acid,* and 9.3 mg of *sodium octyl sulphate,*
- flow rate: 1.7 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 10 µl.

The relative retention times are about 0.8 for atenolol and 1.0 for chlorthalidone.

Inject the reference solution. The test is not valid unless the resolution between atenolol and chlorthalidone peaks is not less than 3.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent for both peaks.

Inject the reference solution and the test solution

Calculate the content of $C_{14}H_{22}N_2O_3$ and $C_{14}H_{11}ClN_2O_4S$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{14}H_{22}N_2O_3$ and not less than 70 per cent of the stated amount of $C_{14}H_{11}ClN_2O_4S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Remove any film coating from the tablets, powder and disperse a quantity of the powder containing 0.1 g of atenolol with 25.0 ml of the mobile phase for 30 minutes with the aid of ultrasound. Filter through a suitable filter (Whatman No 1 is suitable) and use the filtrate.

Reference solution(a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution(b). Dissolve 10.0 mg of *atenolol impurity IPRS* in 0.1 ml of *dimethyl sulfoxide*, with the aid of gentle heat, and dilute to 20.0 ml with the mobile phase.

Reference solution(c). A 0.002 per cent w/v solution of *2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column, 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A mixture of 2 volumes of *tetrahydrofuran*, 18 volumes of *methanol* and 80 volumes of *0.025 M potassium dihydrogen orthophosphate* containing 1.0 g of *sodium octanesulphonate* and 0.4 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml and adjusted to pH 3.0 with *orthophosphoric acid* ,
- flow rate: 2 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 20 µl

Inject reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (b) resembles the reference chromatogram obtained with the *atenolol impurity standard IPRS* and the peaks due to tertiary amine, which is normally a doublet and bis ether are clearly separated. If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase; if its concentration is increases, the retention time of the tertiary amine is prolonged.

Inject reference solution (a), (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-(4-chloro-3-sulfamoyl-benzoyl) benzoic acid is not more than the area of the peak in the chromatogram obtained with reference solution (c) (2 per cent, with reference to the content of chlorthalidone), the area of any peak corresponding to blocker acid is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent, with reference to the content of atenolol) and the area of any peak corresponding to either tertiary amine or bis ether is not more than half of the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent, with reference to the content of atenolol).

Uniformity of content. Complies with the test stated under Tablets. (*For chlorthalidone*)

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in 15 ml of the mobile phase, and disperse with the aid of ultrasound for about 30 minutes, allow to cool, dilute to volume with 25.0 ml with mobile phase shake and filter, rejecting the first few ml of the filtrate. Use the filtrate.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{14}H_{11}ClN_2O_4S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.1 g of atenolol and transfer to a 100.0 ml volumetric flask. Add about 70.0 ml of the mobile phase and disperse with the aid of ultrasound for about 30 minutes, allow to cool, dilute to volume with mobile phase and filter, rejecting the first few ml of filtrate. Dilute 5.0 ml of the solution to 50.0 ml with mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *atenolol IPRS* in mobile phase.

Reference solution (b). A 0.025 per cent w/v solution of *chlorthalidone IPRS* in mobile phase.

Reference solution (c). Transfer 5.0 ml reference solution (a) and 5.0 ml reference solution (b) in 50.0 ml volumetric flask and dilute to volume up to the mark with mobile phase and mix.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 74 volumes of 0.05 per cent w/v solution of *sodium octanesulphonate*, 20 volumes of *acetonitrile*, 5 volumes of *propan-2-ol* and 1 volume of *sulphuric acid* (10 per cent v/v) and adjusted to pH 3.0 with *2 M sodium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 20 µl.

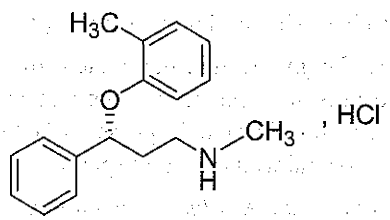
Inject the reference solution(c). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution(c) and the test solution.

Calculate the content of $C_{14}H_{22}N_2O_3$ and $C_{14}H_{11}ClN_2O_4S$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Atomoxetine Hydrochloride



$C_{17}H_{21}NO,HCl$ Mol. Wt. 291.8

Atomoxetine Hydrochloride is (*R*)-*N*-Methyl-3-phenyl-3-(*o*-tolyloxy)propylamine hydrochloride.

Atomoxetine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{21}NO,HCl$ calculated on the anhydrous basis.

Category. Cerebral activator.

Description. A white to creamish yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atomoxetine hydrochloride IPRS* or with the reference spectrum of atomoxetine hydrochloride.

Tests

Specific optical rotation (2.4.22). −42.0° to −36.0°, determined in a 1.0 per cent w/v solution in *methanol.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 12 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.012 per cent w/v solution of *atomoxetine hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving *0.05 M potassium dihydrogen orthophosphate* in *water*, add 2 ml of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid* and 30 volumes of *acetonitrile*,
- flow rate: 1 ml per minute.
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.1 g.

Assay. Dissolve 0.25 g in 40 ml of *glacial acetic acid* and add 10 ml of 5 per cent w/v of *mercuric acetate solution* in *glacial acetic acid.* Titrate with *0.1M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02918 g of $C_{17}H_{21}NO,HCl$.

Atomoxetine Capsules

Atomoxetine Capsules contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atomoxetine, $C_{17}H_{21}NO$.

Usual strengths. 18 mg; 25 mg; 40 mg; 60 mg; 80 mg; 100 mg.

Identification

A. Dissolve the content of capsules containing 60 mg of atomoxetine in 10 ml of *methanol*, centrifuge at 4000 rpm for 5 minutes, Evaporate the solution to a dry powder with the aid of a stream of air or nitrogen. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atomoxetine hydrochloride IPRS* treated in the same manner or with the reference spectrum of atomoxetine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle) (Use sinker, if required),
Medium. 1000 ml of *0.1M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve a quantity of *atomoxetine hydrochloride IPRS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{21}NO$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{21}NO$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and mix the content of 20 capsules. Disperse a quantity of the mixed content containing 0.1 g of atomoxetine in 50 ml of the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. Disperse 0.1 g each of *atomoxetine hydrochloride IPRS* and *urea* in 10 ml of *water*, mix with the aid of ultrasound for 3 minutes, heat at 85° in an oven for 40 minutes, and allow to cool to room temperature. Dilute the resulting solution to 100.0 ml with the mobile phase.

NOTE—The oven temperature and time in the oven can be adjusted to give a suitable level of atomoxetine N-amide peak.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3.5 µm),
- mobile phase: a mixture of 59 volumes of buffer solution prepared by dissolving 4.9 g of *sodium 1-decanesulphonate* and 6.9 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.1 with *orthophosphoric acid* and 41 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 10 µl.

Name	Relative retention time
Desmethyl atomoxetine[1]	0.76
Atomoxetine	1.0
Atomoxetine N-amide[2]	1.2

[1](R)-N-Methyl-3-phenoxy-3-phenylpropan-1-amine.

[2](R)-1-Methyl-1-(3-phenyl-3-(o-tolyloxy)propyl)urea (For system suitability purposes only).

Inject the reference solution. The test is not valid unless the resolution between the peaks due to atomoxetine N-amide and atomoxetine is not less than 2.6.

Inject the test solution. The area of any peak corresponding to desmethyl atomoxetine is not more than 0.3 per cent, the area of any other secondary peak is not more than 0.2 per cent and the sum of the areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and mix the content of 20 capsules. Disperse a quantity of the mixed contents containing 10 mg of atomoxetine in 65 ml of the mobile phase, shake for 20 minutes and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A *solution containing* 0.01 per cent w/v of *atomoxitine hydrochloride IPRS* and 0.002 per cent w/v of *o-cresol* in the mobile phase.

Reference solution (b). A 0.0114 per cent w/v solution of *atomoxitine hyrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 7.5 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3.5 µm),
- column temperature: 35°,
- mobile phase: dilute 3 ml of triethylamine in a solution contaning a mixture of 62 volumes of buffer solution

prepared by dissolving 5.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 38 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The relative retention time with reference to atomoxetine for o-cresol is 1.3.

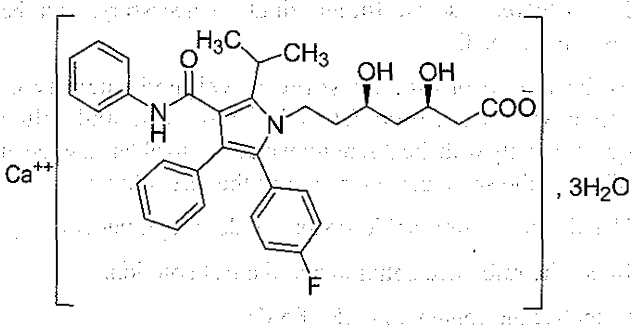
Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to atomoxetine and o-cresol is not less than 3.5, the tailing factor for the principal peak is not more than 2.0 in the chromatogram obtained with reference solution (a) and the relative standard deviation for replicate injections is not more than 1.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_{17}H_{21}NO$ in the capsules.

Storage. Store protected from moisture.

Atorvastatin Calcium



$C_{66}H_{68}CaF_2N_4O_{10},3H_2O$ Mol. Wt. 1209.4

Atorvastatin Calcium is calcium salt of (β*R*,8*R*)-2-(4-fluorophenyl)-α,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid trihydrate.

Atorvastatin Calcium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{66}H_{68}CaF_2N_4O_{10}$, calculated on the anhydrous basis.

Category. Antihyperlipidaemic.

Description. A white to off-white, crystalline powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atorvastatin calcium IPRS* or with the reference spectrum of atorvastatin calcium.

B. Determine by atomic absorption spectro-photometry (2.4.2). A 0.005 per cent w/v solution of the substance under examination in a mixture of 75 volumes of *methanol*, 25 volumes of *water* and 2 volumes of *hydrochloric acid* using air-acetylene flame, shows absorption at the calcium emission line at 422.7 nm.

Tests

Specific optical rotation (2.4.22). −12.0° to −6.0°, determined in a 1.0 per cent w/v solution in *dimethylsulphoxide*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in 10 ml of *methanol* and dilute to 100 ml with the solvent mixture.

Reference solution (a). A 0.5 per cent w/v solution of *atorvastatin calcium IPRS* in *methanol*. Dilute 5 ml of the solution to 50 ml with the solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 92.5 volumes of *acetonitrile* and 7.5 volumes *tetrahydrofuran*,
  B. a mixture of 58 volumes of a buffer solution prepared by dissolving 5.75 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water* and 42 volumes of mobile phase A,
  C. a mixture of 20 volumes of the buffer solution, 20 volumes of mobile phase A and 60 volumes of *methanol*,
- a gradient programme using the conditions given below,
- spectrophotometer set at 246 nm,
- injection volume: 20 µl,
- injection delay 10 minutes.

Time (in min.)	Flow rate (ml per min.)	Mobile phase B (per cent v/v)	Mobile phase C (per cent v/v)
0	1.8	100	0
20	1.8	100	0
35	1.5	25	75
40	1.5	25	75
55	1.5	0	100
60	1.8	100	0

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 1.5.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any individual secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak obtained in the chromatogram obtained in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 6.0 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*.

Test solution. Dissolve 80 mg of the substance under examination in 20 ml of *methanol* and dilute to 200 ml with the solvent mixture. Dilute the solution with the solvent mixture to produce a solution containing 0.008 per cent w/v of Atorvastatin Calcium.

Reference solution. Dissolve 20 mg of *atorvastatin calcium IPRS* in 5 ml of *methanol* and dilute to 50 ml with the solvent mixture. Dilute the solution with the solvent mixture to produce a solution containing 0.008 per cent w/v of Atorvastatin Calcium.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 58 volumes of a buffer solution prepared by dissolving 5.75 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water* and 42 volumes of a mixture of 92.5 volumes of *acetonitrile* and 7.5 volumes of *tetrahydrofuran.*,
- flow rate: 1.8 ml per minute,
- spectrophotometer set at 246 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{66}H_{68}CaF_2N_4O_{10}$.

Storage. Store protected from light at a temperature not exceeding 30°.

Atorvastatin Tablets

Atorvastatin Calcium Tablets

Atorvastatin Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atorvastatin, $C_{33}H_{35}FN_2O_5$.

Usual strengths. 10 mg; 20 mg; 40 mg; 80 mg (1 mg of Atorvastatin Calcium ($C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$) is equivalent to 923.8 µg of atorvastatin $C_{33}H_{35}FN_2O_5$.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *atorvastatin calcium IPRS* in *methanol*, and dilute quantitatively with the dissolution medium to obtain a solution of about the same concentration as the test solution.

Use the chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{33}H_{35}FN_2O_5$.

Q. Not less than 70 per cent of the stated amount of $C_{33}H_{35}FN_2O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*.

Test solution. Weigh a quantity of the powdered tablets containing 50 mg of atorvastatin, disperse in 10 ml of *methanol*, add 20 ml of the solvent mixture, disperse with the aid of ultrasound, if required, and dilute to 100.0 ml with the solvent mixture and filter.

Reference solution (a). Weigh a suitable quantity of *atorvastatin calcium IPRS*, dissolve in 5 ml of *methanol* and dilute to 50.0 ml with the solvent mixture, to produce 0.05 per cent w/v of atorvastatin.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 92.5 volumes of *acetonitrile* and 7.5 volumes *tetrahydrofuran*,
  B. a mixture of 58 volumes of a buffer solution prepared by dissolving 5.75 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water* and 42 volumes of mobile phase A,
  C. a mixture of 20 volumes of the buffer solution, 20 volumes of mobile phase A and 60 volumes of *methanol*,
- a gradient programme using the conditions given below,
- spectrophotometer set at 246 nm,
- injection volume: 20 µl,
- injection delay 10 minutes.

Time (in min.)	Flow rate (ml per min.)	Mobile phase B (per cent v/v)	Mobile phase C (per cent v/v)
0	1.8	100	0
20	1.8	100	0
35	1.5	25	75
40	1.5	25	75
55	1.5	0	100
60	1.8	100	0

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 1.5.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than 4 times the area of the peak in the chromatogram obtained with reference solution (b) (4.0 per cent). Ignore any peak with an area less than 0.05 times the area of the peak obtained with reference solution (b) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay using the following test solution.

Test solution. Disperse one tablet in 3 ml of *water*, add 25 ml of *methanol* and mix with the aid of ultrasound, make up to 50 ml with the solvent mixture, filter. Dilute sufficient amount of the the filtrate with solvent mixture to produce a solution containing 0.008 per cent w/v of atorvastatin.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 0.9 g of *sodium hydroxide* in 1000 ml of *water*, adjusted to pH 6.8 with *phosphoric acid* or *sodium hydroxide*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powdered tablets containing about 80 mg of atorvastatin, add 3 ml to 5 ml of *water* and disperse in sufficient *methanol* to produce a solution containing 0.016 per cent w/v of atorvastatin. Disperse with the aid of ultrasound, if required, and filter. Dilute the filtrate with sufficient of the solvent mixture to produce a solution containing 0.008 per cent w/v of atorvastatin.

Reference solution. Weigh a suitable quantity of *atorvastatin calcium IPRS* and dissolve in sufficient *methanol* to produce a solution containing 0.08 per cent of atorvastatin. To 5 ml of the solution, add 20 ml of *methanol* and dilute to 50 ml with the solvent mixture to produce a solution containing 0.008 per cent w/v of atorvastatin.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 1.54 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 4.0 with *glacial acetic acid*, and 50 volumes of a mixture of 92.5 volumes of *acetonitrile* and 7.5 volumes of *tetrahydrofuran*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 246 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution the test solution.

Calculate the content of $C_{33}H_{35}FN_2O_5$ in the tablets.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of atorvastatin.

Atorvastatin and Fenofibrate Tablets

Atorvastatin Calcium and Fenofibrate Tablets

Atorvastatin and Fenofibrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atorvastatin, $(C_{33}H_{35}FN_2O_5)_2$, and fenofibrate, $C_{20}H_{21}ClO_4$.

Usual strengths. Atorvastatin Calcium equivalent to Atorvastatin, 10 mg and Fenofibrate, 72.5 mg; Atorvastatin, 20 mg and Fenofibrate, 145 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of a solution prepared by dissolving 7.21 g of *sodium lauryl sulphate* in 1000 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), using the chromatographic system described under Assay.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). Weigh accurately about 55 mg of *atorvastatin calcium IPRS* in a 50 ml volumetric flask, add 30.0 ml of *methanol,* sonicate and make up the volume with *methanol.*

Reference solution (b). Weigh accurately about 80 mg of *fenofibrate IPRS* in a 25-ml volumetric flask, add 15 ml of *methanol,* sonicate and make up the volume with *methanol.*

Reference solution (c). Dilute a suitable volume of reference solution (a) and reference solution (b) with dissolution medium to obtain a solution having similar concentration to the test solution.

Inject reference solution (c) and the test solution.

Calculate the content of $(C_{33}H_{35}FN_2O_5)_2$, and $C_{20}H_{21}ClO_4$ in the medium.

Q. Not less than 70 per cent of the stated amounts of $(C_{33}H_{35}FN_2O_5)_2$ and $C_{20}H_{21}ClO_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Use chromatographic system and test solution as described under Assay, except injection volume, use 50 µl.

Inject the test solution. The area of any secondary peak is not more than 1.0 per cent and the sum of the areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalisation.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Disperse 1 tablet in 10 ml of *water* with the aid of ultrasound. Add about 30 ml of *methanol* and disperse with aid of ultrasound for 15 minutes, cool and dilute to 50.0 ml with *methanol* and mix. Centrifuge at 2500 rpm for 10 minutes, rejecting the first few ml of filtrate. Dilute 5.0 ml of the solution to 25.0 ml with the mobile phase.

Reference solution. A 0.08 per cent w/v solution of *atorvastatin IPRS* in *methanol*. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Carry out the chromatographic system described under Assay.

Calculate the content of $(C_{33}H_{35}FN_2O_5)_2$, in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.2 g of fenofibrate into a 250-ml volumetric flask, add 100 ml of *methanol* and sonicate for 25 minutes with intermittent shaking. Make up the volume with *methanol*, mix and centrifuge. Dilute 4.0 ml of clear supernatant to 10.0 ml with mobile phase.

Reference solution (a). Weigh accurately about 60 mg of *atorvastatin calcium IPRS* in a 50-ml volumetric flask, add 30 ml of *methanol,* sonicate it to dissolve and make up the volume with *methanol.*

Reference solution (b). Weigh accurately about 80 mg of *fenofibrate IPRS* in a 25-ml volumetric flask, add 15 ml of *methanol,* sonicate it to dissolve and make up the volume with *methanol.*

Reference solution (c). Dilute a suitable volume of reference solution (a) and reference solution (b) with mobile phase to obtain a solution having similar concentration to the test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C18),
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 30 volumes of *methanol,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between atorvastatin and fenofibrate peaks is not less than 27, the column efficiency is not less than 7000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent for atorvastatin peak.

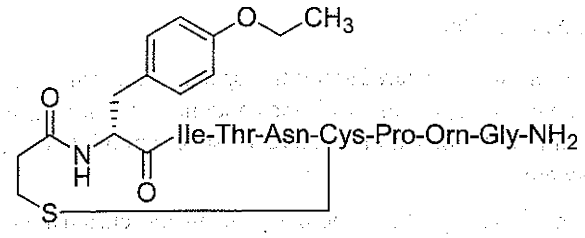
Inject reference solution (c) and the test solution.

Calculate the content of $(C_{33}H_{35}FN_2O_5)_2$, and $C_{20}H_{21}ClO_4$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of atorvastatin, and fenofibrate.

Atosiban Acetate



$C_{43}H_{67}N_{11}O_{12}S_2$ Mol Wt. 994.2

Atosiban Acetate is [1-(3-Sulfanylpropanoyl)-2-(4-*O*-ethyl-*D*-tyrosine)-4-threonine-8-ornithine]oxytocin.

Atosiban Acetate contains not less than 93.0 per cent and not more than 107.0 per cent of $C_{43}H_{67}N_{11}O_{12}S_2$, calculated on the anhydrous and acetic acid free basis.

Category. Oxytocin antagonist.

Description. A white to off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atosiban acetate IPRS* or with the reference spectrum of Atosiban acetate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). – 53.0° to – 43.0°, calculated on anhydrous and acetic acid free basis, determined in 1.0 per cent w/v solution of 1.0 per cent v/v *acetic acid* in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 1.94 g *ammonium acetate* in 1000 ml of *water*, adjusted to pH 4.0 with *trifluoroacetic acid*.

Test solution. Dissolve 20 mg of the substance under examination in mobile phase B and diluted to 5.0 ml with mobile phase B.

Reference solution. A 0.004 per cent w/v solution of *Atosiban acetate IPRS* in mobile phase B.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, such as ZIC HILIC (5 µm),
- column temperature: 60°,
- mobile phase: A . a mixture of 5 volumes of the buffer solution and 95 volumes of *acetonitrile*,
  B. a mixture of 40 volumes of the buffer solution and 60 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 0.7 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
30	70	30
40	60	40
50	60	40
51	85	15
65	85	15

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with test solution the area of any secondary peak is not more than the area of reference solution (1.0 per cent), and the sum of areas of all the secondary peaks is not more than the area of 3 times the area of reference solution (3.0 per cent).

Acetic acid. Not more than 14 per cent w/w on anhydrous basis.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in *water* and dilute to 5.0 ml with *water*.

Reference solution. A 0.07 per cent w/v solution of *acetic acid IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.1 per cent v/v solution of *orthophosphoric acid* in *water* and
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl,

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	99	1
15	99	1
22	5	95
23	99	1
35	99	1

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates , the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of CH_3COOH.

Water (2.3.43). Not more than 15.0 per cent, determined on 0.05g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in mobile phase B and dilute to 25.0 ml with mobile phase B.

Reference solution. A 0.1 per cent w/v solution of *atosiban acetate IPRS* in mobile phase B.

Use chromatographic system as described under Related substances.

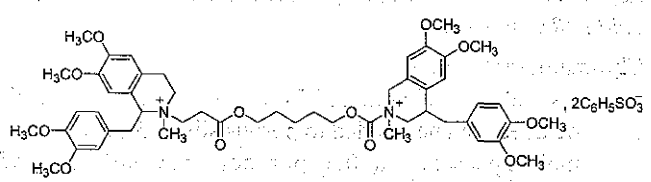
Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{43}H_{67}N_{11}O_{12}S_2$.

Storage. Store protected from moisture, at a temperature between 2° to 8°.

Atracurium Besylate



$C_{65}H_{82}N_2O_{18}S_2$ Mol. Wt.1243.5

Atracurium Besylate is isoquinolinium, 2,2'-[1,5-pentanediyl bis[oxy(3-oxo-3,1-propanediyl)]]bis [1-[(3,4-dimethoxy-phenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-, dibenzenesulphonate.

Atracurium Besylate contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{65}H_{82}N_2O_{18}S_2$, calculated on the anhydrous basis.

It contains not less than 5.0 per cent and not more than 6.5 per cent of the *trans-trans* isomer, not less than 34.5 per cent and not more than 38.5 per cent of the *cis-trans* isomer, and not less than 55.0 per cent and not more than 60.0 per cent of the *cis-cis* isomer.

Category. Neuromuscular blocking agent.

Description. A white to off-white solid.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atracurium besylate IPRS* or with the reference spectrum of atracurium besylate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Methyl benzenesulphonate. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 10.2 g of *monobasic potassium phosphate* in about 950 ml of *water*, adjusted to pH 3.1 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Test solution. Dissolve 0.1 g of Atracurium Besylate in mobile phase A and dilute to 10 ml with mobile phase A.

Reference solution (a). A 0.02 per cent w/v solution of *methyl benzenesulphonate* in *acetonitrile*. Dilute this solution to obtain a 0.0001 per cent w/v solution with mobile phase A.

Reference solution (b). Transfer 1 ml of the test solution and 5 ml of 0.02 per cent w/v solution of *methyl benzenesulphonate* in *acetonitrile* and dilute to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 75 volumes of the buffer solution, 20 volumes of *acetonitrile* and 5 volumes of *methanol*,

  B. a mixture of 50 volumes of the buffer solution, 30 volumes of *methanol* and 20 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 217 nm,
- injection volume: 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
15	75	25
25	55	45
30	55	45
38	0	100
45	0	100
47	80	20

Inject reference solution (a) and (b). The test is not valid unless the resolution between the *trans-trans* isomer and methyl benzenesulphonate is not less than 12.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 12 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to methyl benzenesulphonate is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Toluene. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 20 mg of the substance under examination in 1 ml of *water*.

Reference solution. A 0.01 per cent w/v solution of *toluene* in *water*.

Chromatographic system

- a fused silica column 30 m x 0.53 mm, coated with chemically cross-linked 5 per cent Phenyl- 95 per cent methylpolysiloxane (5 µm),
- temperature:
  column. 35° for 5 minutes, then raised at the rate of 8° per minute to 175°, followed by an increase at a rate of 35° per minute to 260°, and maintained at 260° for at least 16 minutes,
- inlet port. 70° and detector at 260°,
- flame ionization detector,
- linear velocity: 35 cm per second using nitrogen as carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the relative standard deviation of the toluene peak for replicate injections is not more than 15 per cent.

Inject 1 µl of the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to toluene is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 10.2 g of *monobasic potassium phosphate* in 950 ml of *water*, adjusted to pH 3.1 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Test solution. Dissolve 0.1 g of the substance under examination in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (a). A 0.1 per cent w/v solution of *atracurium besylate IPRS* in mobile phase A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 75 volumes of the buffer solution, 20 volumes of *acetonitrile* and 5 volumes of *methanol*,
  B. a mixture of 50 volumes of the buffer solution, 30 volumes of *methanol* and 20 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100
45	0	100
50	80	20

Name	Relative retention time	Correction factor
Atracurium impurity E[1]	0.2	---
Atracurium impurity F[2]	0.25	—
Atracurium impurity G[3]	0.3	0.5
Atracurium impurity D	0.45[4] and 0.5[5]	—
Atracurium *trans-trans* isomer	0.8	---
Atracurium *cis-trans* isomer	0.9	—
Atracurium *cis-cis* isomer	1.0	—
Atracurium impurity A	1.04[6] and 1.08[7]	—
Atracurium impurity I	1.07[8] and 1.12[9]	—
Atracurium impurity H	1.07[10] and 1.12[11]	—
Atracurium impurity K[12]	1.09 and 1.12	---
Atracurium impurity B[13]	1.15	—
Atracurium impurity C	1.2[14] and 1.3[15]	—

[1]3-[1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinio]propanoate,

[2]1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium,

[3]1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline,

[4]trans isomer of 1-(3,4-dimethoxybenzyl)-2-[3-[(5-hydroxypentyl) oxy]-3-oxopropyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium,

[5]cis isomer of 1-(3,4-dimethoxybenzyl)-2-[3-[(5-hydroxypentyl)oxy]-3-oxopropyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium,

[6]cis-trans isomer of 1-(3,4-dimethoxybenzyl)-2-[13-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3,11-dioxo-4,10-dioxatridecyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium,

[7]cis-cis Isomer of 1-(3,4-dimethoxybenzyl)-2-[13-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3,11-dioxo-4,10-dioxatridecyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium,

[8]cis-trans isomer of 2,2'-[(3-methylpentane-1,5)-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium],

[9]cis-cis isomer of 2,2'-[(3-methylpentane-1,5)-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium],

[10]cis-trans isomer of 2,2'-[hexane-1,6-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium],

[11]cis-cis isomer of 2,2'-[hexane-1,6-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium],

[12]2,2'-[(Hexane-1,5)-diylbis(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium],

[13]Pentane-1,5-diyl bis[3-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)propanoate],

[14]trans isomer of 1-(3,4-dimethoxybenzyl)-2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium benzenesulfonate,

[15] cis isomer of 1-(3,4-dimethoxybenzyl)-2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium benzenesulfonate.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to atracurium trans-trans isomer and the cis-trans isomer is not less than 1.5 and the resolution between the peaks due to atracurium cis-trans isomer and the cis-cis isomer is not less than 1.5.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the sum of the areas of the two isomer peaks corresponding to atracurium impurity A and D is not more than 1.5 times the sum of the areas of three principal peaks in the chromatogram obtained with reference solution (b) (1.5 per cent), the area of any peak corresponding to atracurium impurity E is not more than 1.5 times the sum of the areas of three principal peaks in the chromatogram obtained with reference solution (b) (1.5 per cent), the area of the peaks corresponding to atracurium impurity F and G is not more than the sum of the areas of three principal peaks in the chromatogram obtained with reference solution (b) (1.0 per cent), the sum of the areas of the two isomer peaks corresponding to atracurium impurity C, I, H and K is not more than the sum of the areas of three principal in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any other secondary peak is not more than 0.1 times the sum of the areas of three principal peaks in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all the secondary peaks is not more than 3.5 times the sum of the areas of three principal peaks in the chromatogram obtained with reference solution (b) (3.5 per cent). Ignore any peak with an area less than 0.05 times the sum of the areas of three principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 5.0 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 10.2 g of *monobasic potassium phosphate* in about 950 ml of *water*, adjusted to pH 3.1 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Test solution. Dissolve 0.1 g of the substance in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution. A 0.1 per cent w/v solution of *atracurium besylate IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 75 volumes of the buffer solution, 20 volumes of *acetonitrile* and 5 volumes of *methanol*,

  B. a mixture of 50 volumes of the buffer solution, 30 volumes of *methanol* and 20 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100
35	80	20

The relative retention time with reference to *cis-cis* isomer for *trans-trans* isomer is about 0.8 and for *cis-trans* isomer is about 0.9.

Inject the reference solution. The test is not valid unless the resolution between the *trans-trans* isomer and the *cis-trans* isomer and between the *cis-trans* isomer and the *cis-cis* isomer is not less than 1.1 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{65}H_{82}N_2O_{18}S_2$ and measure the responses for the 3 isomeric peaks.

Storage. Store protected from light and moisture at a temperature between 2° to 8°.

Atracurium Besylate Injection

Atracurium Besylate Injection is a sterile solution containing not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of atracurium besylate, $C_{65}H_{82}N_2O_{18}S_2$.

Atracurium Besylate Injection contains an amount of the *trans-trans*-isomer equivalent to not less than 5.0 per cent and not more than 6.5 per cent of the stated amount of atracurium besylate, an amount of the *cis-trans*-isomer equivalent to not less than 34.5 per cent and not more than 38.5 per cent of the stated amount of atracurium besylate and an amount of the *cis-cis*-isomer equivalent to not less than 55.0 per cent and not more than 60.0 per cent of the stated amount of atracurium besylate.

NOTE— The injection is unstable at room teimperature. Store all samples in the refrigerator. Analyze all preparation as soon as possible or use a refrigerated sample.

Usual strength. 10 mg per ml.

Identification

In the Assay, the principal peaks of three isomers of atracurium besylate in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 3.65.

Related substances. Determine by liquid chromatography.

Buffer solution. Dissolve 10.2 g of *monobasic potassium phosphate* in 950 ml of *water*, adjusted to pH 3.1 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Test solution. Dilute a volume of injection containing 50 mg of atracurium besylate in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution. A 0.002 per cent w/v solution of *atracurium besylate IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 75 volumes of the buffer solution, 20 volumes of *acetonitrile* and 5 volumes of *methanol*,

  B. a mixture of 50 volumes of the buffer solution, 30 volumes of *methanol* and 20 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100
45	0	100
50	80	20

Name	Relative retention time	Correction factor
Benzene sulphonic acid	0.08	---
Acidic compound	0.22	---
Laudanosine	0.29	0.5
Hydroxy compound *trans*-isomers	0.44	---
Hydroxy compound *cis*-isomers	0.5	---
Atracurium besylate *trans-trans*-isomer	0.8	---
Atracurium besylate *cis-trans*-isomer	0.9	---
Atracurium besylate *cis-cis*-isomer	1.0	---
Monoacrylate *trans*-isomers	1.28	---
Monoacrylate *cis*- isomers	1.33	---

Inject the reference solution. The test is not valid unless the resolution between the peaks due to atracurium *trans-trans* isomer and the *cis-trans* isomer is not less than 1.5 and the resolution between the peaks due to atracurium *cis-trans* isomer and the *cis-cis* isomer is not less than 1.5.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any

peak corresponding to acidic compound is not more than 3 times the sum of the areas of three principal peaks in the chromatogram obtained with the reference solution (6.0 per cent), the sum of the areas of any peaks corresponding to *cis*- and *trans*-isomers of the hydroxy compound is not more than 3 times the sum of the areas of three principal peaks in the chromatogram obtained with the reference solution (6.0 per cent), and the area of any peak corresponding to laudanosine is not more than 1.5 times the sum of the areas of three principal peaks in the chromatogram obtained with the reference solution (3.0 per cent).The sum of the areas of any peaks corresponding to *cis*- and *trans*-isomers of the monoacrylate is not more than 1.5 times the sum of the areas of three principal peaks in the chromatogram obtained with the reference solution (3.0 per cent). The area of any other unspecified degradation product is not more than 0.05 times the sum of the areas of three principal peaks in the chromatogram obtained with the reference solution (0.1 per cent). The sum of the areas of all the secondary peaks is not more than 7.5 times the sum of the areas of three principal peaks in the chromatogram obtained with the reference solution (15.0 per cent). Ignore the peak due to benzene sulphonic acid.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 5.56 Endotoxin Units per mg of atracurium besylate.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 10.2 g of *monobasic potassium phosphate* in about 950 ml of *water*, adjusted to pH 3.1 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Test solution. Dilute a volume of injection containing 50 mg of Atracurium Besylate in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution. A 0.1 per cent w/v solution of *atracurium besylate IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 75 volumes of the buffer solution, 20 volumes of *acetonitrile* and 5 volumes of *methanol*,

  B. a mixture of 50 volumes of the buffer solution, 30 volumes of *methanol* and 20 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100
45	0	100
50	80	20

Name	Relative retention time
Atracurium besylate *trans-trans*-isomer	0.8
Atracurium besylate *cis-trans*-isomer	0.9
Atracurium besylate *cis-cis*-isomer	1.0

Inject the reference solution. The test is not valid unless the resolution between the *trans-trans* isomer and the *cis-trans* isomer and between the *cis-trans* isomer and the *cis-cis* isomer is not less than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

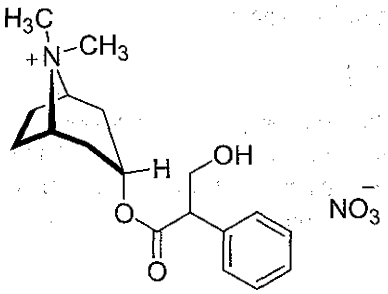
Inject the reference solution and the test solution.

Calculate the content of $C_{65}H_{82}N_2O_{18}S_2$.

Storage. Store protected from light. Preserve in single-dose or multiple-dose containers, preferably of Type I glass, in a refrigerator and protect from freezing.

Atropine Methonitrate

Methylatropine Nitrate



$C_{18}H_{26}N_2O_6$ Mol. Wt. 366.4

Atropine Methonitrate is (*RS*)-(1*R*,3*r*,5*S*)-8-methyl-3-tropoyloxytropanium nitrate.

Atropine Methonitrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{26}N_2O_6$, calculated on the dried basis.

Category. Anticholinergic.

Description. Colourless crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B, C, and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atropine methonitrate IPRS* or with the reference spectrum of atropine methonitrate.

B. To 0.05 ml of a 1 per cent w/v solution add 0.05 ml of a 0.1 per cent w/v solution of *diphenylamine* in *nitrogen-free sulphuric acid*; an intense blue colour is produced.

C. To 2.5 ml of a 10 per cent w/v solution add 2.5 ml of *water* and 2 ml of *dilute sodium hydroxide solution*; no precipitate is produced.

D. Add 1 mg to 4 drops of *fuming nitric acid* and evaporate to dryness on a water-bath; a yellow residue is obtained. To the cooled residue add 2 ml of *acetone* and 4 drops of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

pH (2.4.24). 6.0 to 7.5, determined in a 5.0 per cent w/v solution.

Specific optical rotation (2.4.22). −0.25° to +0.05°, determined in a 10.0 per cent w/v solution, using a 2-dm tube (distinction from hyoscyamine).

Silver. To 10 ml of a 10.0 per cent w/v solution add 0.1 ml of *sodium sulphide solution*. The solution is not more intensely coloured than reference solution BS8 (2.4.1).

Halides (2.3.12). 15 ml of a 5.0 per cent w/v solution in *carbon dioxide-free water* complies with the limit test for chlorides, using 0.3 ml of *chloride standard solution (25 ppm Cl)* for preparing the standard.

Apomethylatropine. A 0.1 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima at about 252 nm and 257 nm (2.4.7). The ratio of the absorbance at about 257 nm to that at about 252 nm is not less than 1.17.

Related substances. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel G*.

Mobile phase. A mixture of 60 volumes of *ethyl acetate*, 15 volumes of *anhydrous formic acid*, 15 volumes of *water* and 10 volumes of *methanol*.

Test solution. A 4.0 per cent w/v solution of the substance under examination in *methanol (90 per cent)*.

Reference solution. Dilute 5 ml of the test solution to 100 ml with *methanol (90 per cent)*, mix and dilute 10 ml of the resulting solution to 100 ml with *methanol (90 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° until the odour of the solvent is not detectable. Allow it to cool to room temperature and spray with *dilute potassium iodobismuthate solution* until spots appear. Any secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

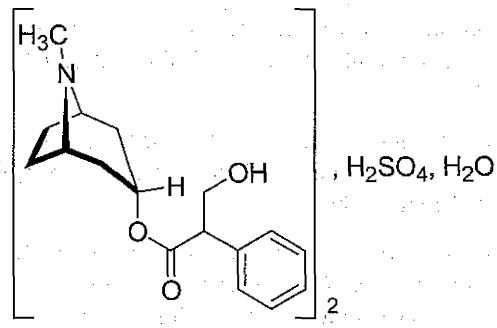
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.3 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03664 g of $C_{18}H_{26}N_2O_6$.

Storage. Store protected from light.

Atropine Sulphate



$(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ Mol. Wt. 694.8

Atropine Sulphate is (*RS*)-(1*R*,3*r*,5*S*)-3-tropoyloxytropanium sulphate monohydrate.

Atropine Sulphate contains not less than 99.0 per cent and not more than 101.0 per cent of atropine sulphate, $(C_{17}H_{23}NO_3)_2$, H_2SO_4, calculated on the anhydrous basis.

Category. Anticholinergic; antidote to cholinesterase inhibitors.

Description. Colourless crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atropine sulphate IPRS* or with the reference spectrum of atropine sulphate.

B. To a 2 per cent w/v solution add *sodium hydroxide solution*, filter and transfer the precipitate with *water*. Dry the precipitate at 60°. To 5 mg of the residue add 5 drops of *fuming nitric acid* and evaporate to dryness on a water-bath. Cool the faintly yellow coloured residue and add 2 ml of *acetone* and 4 drops of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

C. A 5 per cent w/v solution gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.2, determined in a 2.0 per cent w/v solution.

Specific optical rotation (2.4.22). −0.50° to +0.05°, determined in a 10.0 per cent w/v solution, using a 2-dm tube (distinction from hyoscyamine).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 24 mg of the substance under examination in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution (b). A 0.025 per cent w/v solution of *noratropine (atropine impurity A IPRS)* in the test solution. Dilute 5 ml of the solution to 25 ml with mobile phase A.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. dissolve 3.5 g of *sodium lauryl sulphate* in 606 ml of a 0.7 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 3.3 with *0.05 M orthophosphoric acid* and mix with 320 ml of *acetonitrile*,

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
2	95	5
20	70	30
22	95	5

The relative retention time with reference to atropine for atropine impurity A is about 0.89.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to atropine impurity A and atropine is not less than 2.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Apoatropine. Absorbance of a 0.1 per cent w/v solution in *0.01 M hydrochloric acid* at about 245 nm, not more than 0.4 (2.4.7).

Foreign alkaloids and decomposition products. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *acetone*, 7 volumes of *water* and 3 volumes of *strong ammonia solution*.

Test solution. A 2.0 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with *methanol*.

Reference solution (b). Dilute 25 ml of reference solution (a) to 50 ml with *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 15 minutes. Allow it to cool to room temperature and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.5 g.

Assay. Weigh 0.5 g, dissolve in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.06768 g of $(C_{17}H_{23}NO_3)_2,H_2SO_4$.

Storage. Store protected from light.

Atropine Injection

Atropine Sulphate Injection

Atropine Injection is a sterile solution of Atropine Sulphate in Water for Injections.

Atropine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

Usual strengths. 500 µg per ml; 600 µg per ml; 1 mg per ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*.

Test solution. Evaporate a volume of the injection containing 5 mg of Atropine Sulphate to dryness on a water-bath, triturate the residue with 1 ml of *ethanol (95 per cent)*, allow to stand and use the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of *atropine sulphate IPRS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 20 minutes, allow it to cool to room temperature and spray with *potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 5.5.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 55.6 Endotoxin Units per mg of atropine sulphate.

Assay. Determine by liquid chromatography (2.4.14).

For injections containing less than 0.1 per cent w/v of Atropine Sulphate —

Test solution. Use the injection under examination. Inject 100 µl.

Reference solution. A solution containing *atropine sulphate IPRS* and *homatropine hydrobromide IPRS* in the mobile phase, both at the same concentration as the solution under examination. Inject 100 µl.

For injections containing 0.1 per cent w/v or more of Atropine Sulphate -

Test solution. Dilute the injection, if necessary, to obtain 0.1 per cent w/v of Atropine Sulphate with *water*. Inject 20 µl.

Reference solution. A solution containing 0.1 per cent w/v each of *atropine sulphate IPRS* and *homatropine hydrobromide IPRS* in the mobile phase. Inject 20 µl.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a solution containing *0.01 M sodium acetate* and 0.005 M *dioctyl sodium sulphosuccinate* in *methanol (60 per cent)*, adjusted to pH 5.5 with *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 257 nm.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to atropine sulphate and homatropine hydrobromide is not less than 2.5.

Inject the reference solution and the test solution.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the injection.

Storage. Store protected from light.

Atropine Eye Ointment

Atropine Sulphate Eye Ointment

Atropine Eye Ointment is a sterile preparation of Atropine Sulphate in an eye ointment base.

Atropine Eye Ointment contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

Usual strength. 1.0 per cent w/w.

Identification

Determine by thin- layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*.

Test solution. Dissolve a quantity of the ointment containing 10 mg of Atropine Sulphate as completely as possible in 10 ml of *light petroleum (40° to 60°)* and extract with two quantities, each of 10 ml, of *0.05 M sulphuric acid*, washing each acid solution with the same 5 ml of *light petroleum (40° to 60°)*. Mix the acid solutions, make alkaline with *dilute ammonia solution*, and extract with two quantities, each of 15 ml, of *chloroform*. Remove the *chloroform* and dissolve the residue in 2 ml of *ethanol (95 per cent)*.

Reference solution. A 0.5 per cent w/v solution of *atropine sulphate IPRS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 20 minutes, allow it to cool to room temperature and spray with *potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Eye Ointments.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the eye ointment containing about 10 mg of Atropine Sulphate in 10 ml of *ether* and extract with two 10 ml quantities of *0.01 M hydrochloric acid*. Use the combined extracts.

Reference solution. A solution containing 0.05 per cent w/v each of *atropine sulphate IPRS* and *homatropine hydrobromide IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a solution containing *0.01 M sodium acetate* and 0.005 M *dioctyl sodium sulphosuccinate* in *methanol (60 per cent)*, adjusted to pH 5.5 with *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 257 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to atropine sulphate and homatropine hydrobromide is not less than 2.5.

Inject the reference solution and the test solution.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the eye ointment.

Storage. Store at a temperature not exceeding 30°.

Atropine Tablets

Atropine Sulphate Tablets

Atropine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

Identification

A. Determine by thin- layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*.

Test solution. Disperse a quantity of the powdered tablets containing 5 mg of Atropine Sulphate with 1 ml of *ethanol (95 per cent)*, centrifuge and use the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of *atropine sulphate IPRS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 20 minutes, allow it to cool to room temperature and spray with *potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. The powdered tablets give the reactions of sulphates (2.3.1).

Tests

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse one tablet in 2 ml of the mobile phase with the aid of ultrasound, filter.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 30 mg of atropine in 100.0 ml of the mobile phase.

Reference solution. A solution containing 0.03 per cent w/v each of *atropine sulphate IPRS* and *homatropine hydrobromide IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a solution containing *0.01 M sodium acetate* and *0.005 M dioctyl sodium sulphosuccinate* in *methanol* (60 per cent), adjusted to pH 5.5 with *glacial acetic acid*,

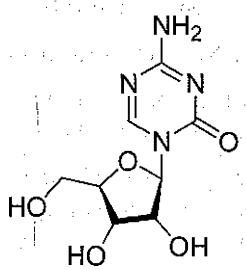
- flow rate: 2 ml per minute,
- spectrophotometer set at 257 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to atropine sulphate and homatropine hydrobromide is not less than 2.5.

Inject the reference solution and the test solution.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the tablet.

Azacitidine



$C_8H_{12}N_4O_5$ Mol Wt. 244.2

Azacitidine is 4-amino-1-β-*D*-ribofuranosyl-1,3,5-triazin-2(1*H*)-one.

Azacitidine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_{12}N_4O_5$, calculated on the dried basis.

Category. Anticancer.

Description. A white to off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *azacitidine IPRS* or with reference spectrum of azacitidine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). + 6.0° to + 10.0°, calculated on as is basis and determined in 2.0 per cent w/v solution in *dimethyl sulphoxide*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in *dimethyl sulphoxide* and dilute to 10.0 ml with *dimethyl sulphoxide*.

Reference solution. A 0.01 per cent w/v solution of *azacitidine IPRS* in *dimethyl sulphoxide*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, (5 µm) (Such as HILIC),
- mobile phase: a mixture of 20 volumes of buffer solution prepared by dissolving 0.77 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 4.0 with *acetic acid*, 180 volumes of *methanol* and 800 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0.

Inject the reference solution and test solution. In the chromatogram obtained with test solution the the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° under vacuum for 3 hour.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in *dimethyl sulphoxide* and dilute to 25.0 ml with *dimethyl sulphoxide*.

Reference solution. A 0.08 per cent w/v solution of *azacitidine IPRS* in *dimethyl sulphoxide*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 10°,
- mobile phase: dissolve 1.54 g of *ammonium acetate* in 1000 ml of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1200 theoretical plates

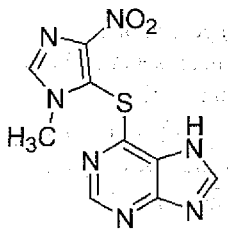
and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{12}N_4O_5$.

Storage. Store protected from moisture, at a temperature between 15° to 30°.

Azathioprine



$C_9H_7N_7O_2S$ Mol. Wt. 277.3

Azathioprine is 6-[(1-methyl-4-nitro-1*H*-imidazol-5yl) sulphanyl]-7*H*-purine.

Azathioprine contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_7N_7O_2S$, calculated on the dried basis.

Category. Immunosuppressant.

Description. A pale-yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *azathioprine IPRS* or with the reference spectrum of azathioprine.

Tests

Acidity or alkalinity. To 0.5 g add 25 ml of *carbon dioxide free water*, shake for 15 minutes and filter. To 20 ml of the filtrate add 0.1 ml of *methyl red solution*. Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

5-Chloro-1-methyl-4-nitroimidazole and 6-mercaptopurine. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose GF254*.

Mobile phase. *Butanol* saturated with *dilute ammonia solution*.

NOTE – Prepare the following solutions immediately before use.

Test solution. Dissolve 0.2 g of the substance under examination in *dilute ammonia solution* and add sufficient *dilute ammonia solution* to produce 10 ml.

Reference solution (a). A 0.02 per cent w/v solution of *chloromethylnitroimidazole IPRS* in *dilute ammonia solution*.

Reference solution (b). A 0.02 per cent w/v solution of *mercaptopurine* in *dilute ammonia solution*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 50° and examine under ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spots corresponding to chloromethylnitroimidazole and mercaptopurine are not more intense than the spots in the chromatograms obtained with reference solution (a) (1.0 per cent) and reference solution (b) (1.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Dissolve 0.25 g in 25 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02773 g of $C_9H_7N_7O_2S$.

Storage. Store protected from light.

Azathioprine Tablets

Azathioprine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of azathioprine, $C_9H_7N_7O_2S$.

Usual strength. 50 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F 254*.

Mobile phase. A mixture of *butan-1-ol* saturated with *6 M ammonia*.

Test solution. Disperse a quantity of the powdered tablets containing 0.2 g of Azathioprine with 50 ml of *6 M ammonia*, filter through a glass micro fibre paper (such as Whatman GF/C) and use the filtrate.

Reference solution. A 0.4 per cent w/v solution of *azathioprine IPRS* in *6 M ammonia*.

Apply to the plate 5 µl of each solution. After removal of the plate, dry the plate at 50° and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with

the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Heat a quantity of the powdered tablets containing 20 mg of Azathioprine with 100 ml of *water* and filter. To 5 ml of the filtrate add 1 ml of *hydrochloric acid* and 10 mg of *zinc powder* and allow to stand for 5 minutes; a yellow colour is produced. Filter, cool in ice, add 0.1 ml of a 10 per cent w/v solution of *sodium nitrite* and 0.1 g of *sulphamic acid* and shake until the bubbles disappear. Add 1 ml of *2-naphthol solution*; a pale pink precipitate is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium: 900 ml of *water*,
Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium, if necessary. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of azathioprine, $C_9H_7N_7O_2S$ in the medium from the absorbance obtained from a solution of known concentration of *azathioprine IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_9H_7N_7O_2S$.

5-Chloro-1-methyl-4-nitroimidazole and 6-mercaptopurine. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose F 254*.

Mobile phase. A mixture of *butan-1-ol* saturated with *6 M ammonia*.

Test solution. Disperse a quantity of the powdered tablets containing 0.2 g of Azathioprine with 10 ml of *6 M ammonia* and filter through a glass micro fibre filter paper (such as Whatman GF/C).

Reference solution (a). A solution containing 2.0 per cent w/v of *azathioprine IPRS* and 0.02 per cent w/v of *6-mercaptopurine* in *6 M ammonia*.

Reference solution (b). A 0.02 per cent w/v solution of *6-mercaptopurine* in *6 M ammonia*.

Reference solution (c). A 0.02 per cent w/v solution of *chloromethylnitroimidazole IPRS* in *6 M ammonia*.

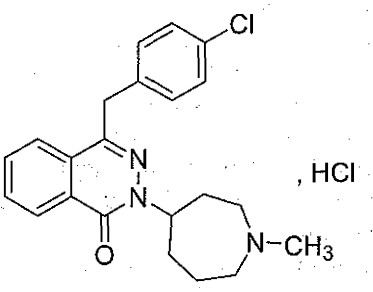
Apply to the plate 5 µl of each solution. After removal of the plate, dry the plate at 50° and examine under ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution corresponding to 6-mercaptopurine in the chromatogram obtained with reference solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). Any spot corresponding to 5-chloro-1-methyl-4-nitroimidazole in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.15 g of Azathioprine with 20 ml of *dimethyl sulphoxide* for 15 minutes and dilute to 500.0 ml with *0.1 M hydrochloric acid*, filter. Dilute 25.0 ml of the filtrate to 1000.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7) using *0.1 M hydrochloric acid* as the blank. Calculate the content of $C_9H_7N_7O_2S$ using a solution of *azathioprine IPRS* of the same concentration in *0.1 M hydrochloric acid*.

Storage. Store protected from light.

Azelastine Hydrochloride



$C_{22}H_{24}ClN_3O,HCl$ Mol. Wt.418.4

Azelastine Hydrochloride is (*RS*)-4-(4-Chlorobenzyl)-2-(1-methylazepan-4-yl)phthalazin-1(2*H*)-one hydrochloride.

Azelastine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{22}H_{24}ClN_3O,HCl$, calculated on the dried basis.

Category. Antihistamine.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *azelastine hydrochloride IPRS* or with the reference spectrum of azelastine hydrochloride.

B. Solution A gives reaction (A) of chlorides (2.3.1).

Tests

Solution A. A 1.0 per cent w/v solution in *carbon dioxide-free water*.

Appearance of solution. Solution A is clear and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A, add 0.2 ml of *bromothymol blue solution*. Not more than 0.1 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 45 volumes of *acetonitrile* and 55 volumes of *water*.

Test solution. Dissolve 0.125 g of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.005 per cent w/v each of *azelastine impurity B IPRS*, *azelastine impurity D IPRS* and *azelastine impurity E IPRS* in the test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups bonded to porous silica (10 µm),
- mobile phase: dissolve 2.16 g of *sodium octanesulphonate* and 0.68 g of *potassium dihydrogen phosphate* in 740 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, add 260 ml of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Azelastine impurity A[1]	0.2	—
Azelastine impurity B[2]	0.3	—
Azelastine impurity C[3]	0.4	—
Azelastine impurity D[4]	0.6	0.7
Azelastine (retention time: about 8 to 9 minutes)	1.0	—
Azelastine impurity E[5]	1.4	2.1

[1] benzohydrazide,

[2] 1-benzoyl-2-[(4*RS*)-1-methylhexahydro-1*H*-azepin-4-yl]diazane,

[3] 2-[(4-chlorophenyl)acetyl]benzoic acid,

[4] 4-(4-chlorobenzyl)phthalazin-1(2*H*)-one,

[5] 3-(4-chlorobenzylidene)isobenzofuran-1(3*H*)-one.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to azelastine impurities B and D is not less than 4.0 and the peaks due to azelastine impurities D and E are baseline separated from the principal peak.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. *NOTE — In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached.*

Dissolve 0.3 g in 5 ml of *anhydrous formic acid*. Add 30 ml of *acetic anhydride*. Titrate quickly with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1.0 ml of *0.1 M perchloric acid* is equivalent to 0.04184 g of $C_{22}H_{25}ClN_3O$.

Azelastine Eye Drops

Azelastine Hydrochloride Eye Drops

Azelastine Eye Drops is a sterile solution of Azelastine Hydrochloride in purified water.

Azelastine Eye Drops Contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of azelastine hydrochloride, $C_{22}H_{24}ClN_3O,HCl$.

Usual strength. 0.5 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.5 to 6.5.

Light absorption. The absorbance of the eye drop at 420 nm (2.4.7) is not more than 0.1.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay.

Inject the test solution. The sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the eye drops containing 2.5 mg of Azelastine Hydrochloride to 50.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *azelastine hydrochloride IPRS* in the mobile phase.

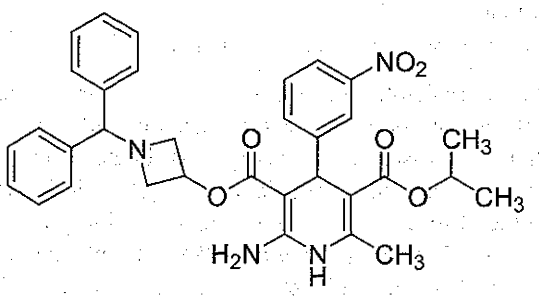
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with cyanopropyl groups bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *water*, 50 volumes of *acetonitrile*, 0.4 volume of *triethylamine* and 0.2 volume of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}ClN_3O,HCl$. in the eye drops.

Azelnidipine



$C_{33}H_{34}N_4O_6$ Mol. Wt. 582.7

Azelnidipine is 3-[1-(Diphenylmethyl)azetidin-3-yl] 5-(1-methylethyl)(4RS)-2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Azelnidipine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{33}H_{34}N_4O_6$, calculated on the dried basis.

Category. Calcium channel blocker

Description. A light yellow to yellow crystalline powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared adsorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *azelnidipine IPRS* or with the reference spectrum of azelnidipine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *acetonitrile* and 20 volumes of *water.*

Test solution. Dissolve 100 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 3.0 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water,* adjusted to pH 5.5 with *orthophosphoric acid,* 45 volumes of *acetonitrile* and 20 volumes of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume:10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 15000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak for the test solution. The area of any secondary peak eluting at a relative retention time of about 0.5 is not more than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of any secondary peak eluting at a relative retention time of about 1.42 is not more than 0.3 times the area of the principal peak in the chromatogram obtained the with the reference solution (0.3 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not

more than 0.7 times the area of the principal peak in the chromatogram obtained with the reference solution (0.7 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying under vacuum at 70° for 5 hours.

Assay. Determine by liquid chromatography (2.4.14)

Solvent mixture. Equal volumes of *water* and *acetonitrile.*

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.002 per cent w/v solution of *azelnidipine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a *0.03M potassium dihydrogen orthophosphate* in *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 256 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
12	30	70
20	30	70
25	80	20
30	80	20

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{33}H_{34}N_4O_6$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Azelnidipine Tablets

Azelnidipine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of azelnidipine, $C_{33}H_{34}N_4O_6$.

Usual strengths. 8 mg; 16 mg.

Identification

A. Weigh a quantity of the powdered tablets containing 4 mg of Azelnidipine, disperse in 150.0 ml of *anhydrous ethanol*, with the aid of ultrasound for 15 minutes with shaking and dilute to 200.0 ml with *anhydrous ethanol*, centrifuge and filter. When examined in the range 200 nm to 400 nm (2.4.7), the resulting solution shows absorption maxima between 253 nm and 257 nm and between 339 nm and 346 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

NOTE — Perform the tests and assay in subdued light and use low-actinic glassware.

Apparatus No. 2 (Paddle),
Medium. 900 ml of *hydrochloric acid* buffer solution pH 1.2 prepared by dissolving 2.0 g of *sodium chloride* in 7.0 ml of *hydrochloric acid* and 500.0 ml of *water*, and diluted to 1000 ml with *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 270 nm (2.4.7). Calculate the content of azelnidipine, $C_{33}H_{34}N_4O_6$ in the medium from the absorbance obtained from a solution prepared by dissolving 45 mg of *azelnidipine IPRS in* 10 ml of *anhydrous ethanol* and diluted to 25.0 ml with same solvent. Dilute 1.0 ml of the solution to 200.0 ml with the dissolution medium.

Q. Not less than 75 per cent of the stated amount $C_{33}H_{34}N_4O_6$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *acetonitrile* and 20 volumes of *water.*

Test Solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 10 mg of Azelnidipine, disperse in 5 ml of solvent mixture with the aid of ultrasound for 15 minutes with intermediate shaking and dilute to 10.0 ml with solvent mixture and centrifuge.

Reference solution (a). A 0.002 per cent w/v solution of *azelnidipine IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the reference solution (a) to 20.0 ml with solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 3.0 g *potassium dihydrogen orthophosphate* in 1000 ml of *water* and 65 volumes of solvent mixture prepared by mixing 70 volumes of *acetonitrile* and 30 volumes of *methanol*, adjusted to pH 5.5 with *orthophosphoric acid*,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The retention time of azelnidipine is about 36 minutes.

Inject reference solution (a) and (b). The test is not valid unless the column efficiency is not less than 15000 theoretical plates, the tailing factor is not more than 1.5 and relative standard deviation for replicate injections is not more than 1.0 per cent with reference solution (a) and the area of principal peak in the chromatogram obtained reference solution (b) is between 3.5 to 6.5 per cent of the area of principal peak in chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with test solution, the area of any secondary peak eluting with an relative retention time of about 0.10 is not more than 0.45 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.9 per cent), the area of any secondary peak eluting with an relative retention time of about 0.13 is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent), the area of any secondary peak eluting with an relative retention time of about 0.50 is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent) and the area of any secondary peak eluting with an relative retention time of about 1.42 is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent). The area of any other secondary is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The sum of areas of all the secondary peaks is not more than 1.75 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.5 per cent).

Uniformity of content. Complies with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in sufficient quantity of reference solution (a) and sufficient volume of solvent mixture with the aid of ultrasound for 10 minutes with intermediate shaking, centrifuge and dilute supernatant solution to obtain a solution containing 0.005 per cent w/v of azelnidipine and 0.0025 w/v of *2,2'-dinaphthylether*.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{33}H_{34}N_4O_6$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *acetonitrile* and 20 volumes of *water.*

Test Solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of Azelnidipine, disperse in 25.0 ml of reference solution (a) and 50 ml solvent mixture with the aid of ultrasound for 10 minutes with intermediate shaking and dilute to 100.0 ml with solvent mixture and mix. Centrifuge this solution and dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *2, 2'-dinaphthylether* in the solvent mixture.

Reference solution (b). A 0.005 per cent w/v solution of *azelnidipine IPRS* and 0.0025 per cent w/v solution of reference solution (a) in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature: 40°,
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 3.0 g of *potasium dihydrogen orthophosphate* in 1000 ml of *water* and 70 volumes of *acetonitrile,* adjusted to pH 6.0 with *dilute sodium hydroxide solution*,
- flow rate: 1ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

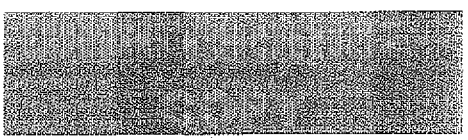
The retention time of azelnidipine and *2, 2'- dinaphthylether* are about 13 minutes and about 25 minutes respectively.

Inject reference solution (b). The test is not valid unless the resolution between azelnidipine and *2, 2'- dinaphthylether* is not less than 12.0 and the relative standard deviation for *2, 2'-dinaphthylether* and azelnidipine is not more than 1.0 per cent.

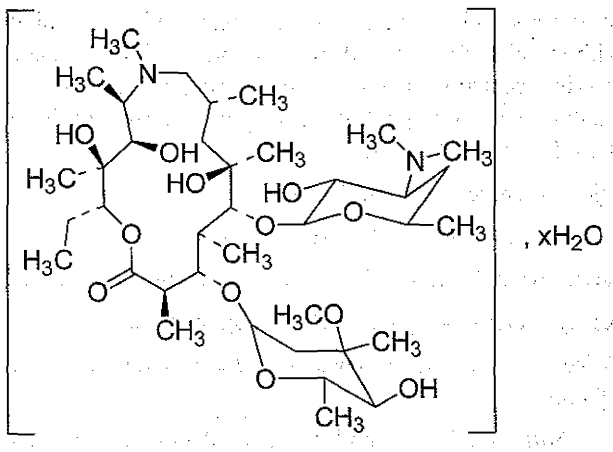
Inject reference solution (b) and the test solution.

Calculate the content of $C_{33}H_{34}N_4O_6$ in the tablets.

Storage. Store protected from light and moisture.



Azithromycin



$C_{38}H_{72}N_2O_{12}.xH_2O$ with x = 1 or 2 Mol. Wt. 749.0 (anhydrous)

Azithromycin is (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one monohydrate or dihydrate.

Azithromycin contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{38}H_{72}N_2O_{12}$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *azithromycin IPRS* or with the reference spectrum of azithromycin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. Dissolve 0.5 g in *anhydrous ethanol* and dilute to 50.0 ml with the same solvent (solution A). Solution A is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 9.0 to 11.0 determined in a solution prepared by dissolving 0.1 g in 25.0 ml of *methanol* and further diluting to 50.0 ml with *carbon dioxide-free water*.

Specific optical rotation (2.4.22). −49.0° to −45.0°, determined in solution A, at 20°.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Prepare a 0.173 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 10.0 with *strong ammonia solution*. To 35 ml of the solution add 30 ml of *acetonitrile* and 35 ml of *methanol*. Mix well.

Test solution. Dissolve 0.2 g of the substance under examination in the solvent mixture and dilute to 25 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *6-demethyl-azithromycin IPRS (azithromycin impurity A)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped octadecylsilane amorphous organosilica polymer (5 µm),
- column temperature: 60°,
- mobile phase: A. a 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate* with the pH adjusted to 8.9 with *dilute phosphoric acid* or with *dilute sodium hydroxide solution*,
  B. a mixture of 250 volumes of *methanol* and 750 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

Name	Relative retention time	Correction factor
Azithromycin impurity L[1]	0.29	2.3
Azithromycin impurity M[2]	0.37	0.6
Azithromycin impurity E[3]	0.43	—
Azithromycin impurity F[4]	0.51	0.3
Azithromycin impurity D[5]	0.54	—
Azithromycin impurity J[6]	0.54	—
Azithromycin impurity I[7]	0.61	—
Azithromycin impurity C[8]	0.73	—
Azithromycin impurity N[9]	0.76	0.7
Azithromycin impurity H[10]	0.79	0.1
Azithromycin impurity A[11]	0.83	—

Azithromycin impurity P	0.92	—
Azithromycin (Retention time: about 45-50 minutes)	1.0	—
Azithromycin impurity O[12]	1.23	—
Azithromycin impurity G[13]	1.26	0.2
Azithromycin impurity B[14]	1.31	—

[1]azithromycin 3′- *N*-oxide,
[2]3′-(*N*,*N*-didemethyl)-3′-*N*-formylazithromycin,
[3]aminoazithromycin,
[4]3′-*N*-demethyl-3′-*N*- formylazithromycin,
[5]14-demethyl-14-(hydroxymethyl)azithromycin,
[6]13-*O*-decladinosylazithromycin,
[7]3′-*N*-demethylazithromycin,
[8]3″-*O*-demethylazithromycin,
[9]3′-de(dimethylamino)-3′-oxoazithromycin,
[10]3′-*N*-[[4-(acetylamino)phenyl]sulfonyl]-3′-*N*-demethylazithromycin
[11]6-demethylazithromycin,
[12]2-desethyl-2-propylazithromycin,
[13]3′-*N*-demethyl-3′-*N*-[(4-methylphenyl)sulfonyl]azithromycin,
[14]3-deoxyazithromycin.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak eluting with relative retention time of about 1.3 due to 3-deoxyazithromycin (azithromycin impurity B) is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). The sum of the areas of all the other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) ;ignore the peaks eluting before azithromycin impurity L and after azithromycin impurity B; ignore the peaks eluting before azithromycin impurity L and after azithromycin impurity B.

Heavy metals (2.3.13). 0.8 g complies with the limit test for heavy metals, Method B (25 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). 1.8 per cent to 6.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*.

Test solution. Dissolve 0.1 g of the substance under examination in the solvent mixture and dilute to 100 ml with the solvent mixture.

Reference solution (a) . A 0.1 per cent w/v solution of *azithromycin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *azithromycin impurity A IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with end capped polar embedded octadecylsilyl amorphous organosilica polymer (5 µm) (Such as Waters Xterra),
- column temperature: 70°,
- mobile phase: a mixture of 10 volumes of 3.484 per cent w/v solution of *dipotassium hydrogen phosphate* previously adjusted to pH 6.5 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 100 µl.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$.

Storage. Store protected from moisture.

Azithromycin Capsules

Azithromycin Capsules contain not less than 90.0 percent and not more than 110.0 percent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

Usual strength. 500 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a buffer solution prepared by adding to 6 litres of *0.1 M dibasic sodium phosphate* about 40 ml of

hydrochloric acid to adjusted to pH 6.0, adding 600 mg of *trypsin* and mixing.

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

Test solution. Use the filtrate, dilute if necessary, with the mobile phase.

Reference solution. A solution of *azithromycin IPRS* in the dissolution medium suitably diluted with the mobile phase to obtain a solution having the same concentration as that of the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{38}H_{72}N_2O_{12}$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Prepare a 0.173 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 10.0 with *strong ammonia solution*. Transfer 35 ml of the solution, add 30 ml of *acetonitrile* and 35 ml of *methanol*. Mix well.

Test solution. Dissolve a suitable weighed quantity of the mixed contents of 20 capsules containing about 0.2 g of Azithromycin in the solvent mixture by shaking mechanically, dilute to 25.0 ml with the solvent mixture and filter.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *6-demethyl-azithromycin IPRS (azithromycin impurity A IPRS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-cappped octadecylsilyl amorphous organosilica polymer for mass spectrometry (5µm) (Such as Waters Xterra),
- column temperature: 60°,
- mobile phase: A. a solution of 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate* with the pH adjusted to 8.9 with *dilute phosphoric acid* or with *dilute sodium hydroxide solution*,

  B. a mixture of 25 volumes of *methanol* and 75 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Time (in min.)	mobile phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

Name	Relative retention time	Correction factor
Azithromycin impurity L[1]	0.29	2.3
Azithromycin impurity M[2]	0.37	0.6
Azithromycin impurity E[3]	0.43	—
Azithromycin impurity F[4]	0.51	0.3
Azithromycin impurity D[5]	0.54	—
Azithromycin impurity J[6]	0.54	—
Azithromycin impurity I[7]	0.61	—
Azithromycin impurity C[8]	0.73	—
Azithromycin impurity N[9]	0.76	0.7
Azithromycin impurity H[10]	0.79	0.1
Azithromycin impurity A[11]	0.83	—
Azithromycin impurity P	0.92	—
Azithromycin (Retention time: about 45-50 minutes)	1.0	—
Azithromycin impurity O[12]	1.23	—
Azithromycin impurity G[13]	1.26	0.2
Azithromycin impurity B[14]	1.31	—

[1]azithromycin 3′- *N*-oxide,
[2]3′-(*N*,*N*-didemethyl)-3′-*N*-formylazithromycin,
[3]aminoazithromycin,
[4]3′-*N*-demethyl-3′-*N*- formylazithromycin,
[5]14-demethyl-14-(hydroxymethyl)azithromycin,
[6]13-*O*-decladinosylazithromycin,
[7]3′-*N*-demethylazithromycin,
[8]3″-*O*-demethylazithromycin,
[9]3′-de(dimethylamino)-3′-oxoazithromycin,
[10]3′-*N*-[[4-(acetylamino)phenyl]sulfonyl]-3′-*N*-demethylazithromycin,
[11]6-demethylazithromycin,
[12]2-desethyl-2-propylazithromycin,
[13]3′-*N*-demethyl-3′-*N*-[(4-methylphenyl)sulfonyl]azithromycin,
[14]3-deoxyazithromycin.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak eluting with an relative retention time of about 1.3 due to 3-deoxyazithromycin (azithromycin impurity B) is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). The sum of the areas of all the other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); ignore the peaks eluting before azithromycin impurity L and after azithromycin impurity B.

Water (2.3.43). Not more than 5.0 per cent determined on 0.2 g of the contents of the capsules.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*.

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing about 0.1 g of Azithromycin, dissolve in about 75 ml of the solvent mixture by shaking mechanically, dilute to 100 ml with the solvent mixture and filter.

Reference solution(a). A 0.1 per cent w/v solution of *azithromycin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *6-demethyl-azithromycin IPRS (azithromycin impurity A IPRS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped polar embedded octadecylsilyl amorphous organosilica polymer (5 µm) (Such as Waters Xterra),
- column temperature: 70°,
- mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of *dipotassium hydrogen phosphate*, previously adjusted to pH 6.5 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 100 µl.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromcyin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the capsules.

Storage. Store protected from moisture.

Azithromycin Eye Drops

Azithromycin Eye Drops is a sterile solution of Azithromycin in a suitable oily vehicle.

Azithromycin Eye Drops contain not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

Usual strength. 1.0 per cent w/v.

Identification

A. Mix a quantity of the eye drops containing 100 mg of Azithromycin with 10 ml of *ethanol*. Allow to stand, retain the upper ethanolic layer and evaporate it to dryness under a stream of nitrogen. Wash the residue with 10 ml of *hexane* followed by a further 50 ml of *hexane* and allow to dry in air. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *azithromycin IPRS* treated in the same manner or with the reference spectrum of azithromycin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. 20 volumes of *dichloromethane* and 80 volumes of *methanol*.

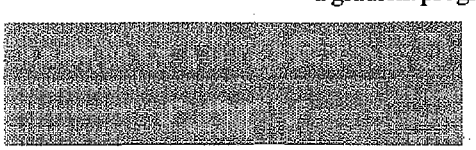
Test solution. Dilute a suitable volume of the eye drops with the solvent mixture to obtain a solution containing 0.8 per cent w/v of Azithromycin.

Reference solution (a). A 0.008 per cent w/v solution of *azithromycin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *6-demethyl-azithromycin IPRS (azithromycin impurity A)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-capped octadecylsilane amorphous organosilica polymer (5 µm) (Such as X-terra MS),
- column temperature: 60°,
- mobile phase: A. a 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate*, adjusted to pH 8.9 with *dilute orthophosphoric acid* or with *dilute sodium hydroxide solution*,
  B. a mixture of 25 volumes of *methanol* and 75 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,



– flow rate: 1 ml per minute,
– spectrophotometer set at 210 nm,
– injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

Name	Relative retention time	Correction factor
Azithromycin impurity L[1]	0.29	2.3
Azithromycin impurity M[2]	0.37	0.6
Azithromycin impurity E[3]	0.43	—
Azithromycin impurity F[4]	0.51	0.3
Azithromycin impurity D[5]	0.54	—
Azithromycin impurity J[6]	0.54	—
Azithromycin impurity I[7]	0.61	—
Azithromycin impurity C[8]	0.73	—
Azithromycin impurity N[9]	0.76	0.7
Azithromycin impurity H[10]	0.79	0.1
Azithromycin impurity A[11]	0.83	—
Azithromycin impurity P	0.92	—
Azithromycin (Retention time: about 45-50 minutes)	1.0	—
Azithromycin impurity O[12]	1.23	—
Azithromycin impurity G[13]	1.26	0.2
Azithromycin impurity B[14]	1.31	—

[1]azithromycin 3'- *N*-oxide,
[2]3'-(*N*,*N*-didemethyl)-3'-*N*-formylazithromycin,
[3]aminoazithromycin,
[4]3'-*N*-demethyl-3'-*N*- formylazithromycin,
[5]14-demethyl-14-(hydroxymethyl)azithromycin,
[6]13-*O*-decladinosylazithromycin,
[7]3'-*N*-demethylazithromycin,
[8]3''-*O*-demethylazithromycin,
[9]3'-de(dimethylamino)-3'-oxoazithromycin,
[10]3'-*N*-[[4-(acetylamino)phenyl]sulfonyl]-3'-*N*-demethylazithromycin,
[11]6-demethylazithromycin,
[12]2-desethyl-2-propylazithromycin,
[13]3'-*N*-demethyl-3'-*N*-[(4-methylphenyl)sulfonyl]azithromycin,
[14]3-deoxyazithromycin.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak eluting with relative retention time of about 1.3 due to 3-deoxyazithromycin (azithromycin impurity B) is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent) and the sum of the areas of all other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). Ignore the peaks eluting before azithromycin impurity L and after azithromycin impurity B.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *dichloromethane* and 80 volumes of *methanol*.

Test solution. Dilute a volume of eye drops containing 0.05 g of Azithromycin to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *azithromycin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.05 per cent w/v, each of, *azithromycin IPRS* and *azithromycin impurity A IPRS* in the solvent mixture.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane vinyl polymer (5µm) (Such as Asahipak ODP-50),
– column temperature: 40°,
– mobile phase: a mixture of 40 volumes of 0.67 per cent w/v solution of *dipotassium hydrogen orthophosphate*, adjusted to pH 11 with a 56 per cent w/v solution of *potassium hydroxide* and 60 volumes of *acetonitrile*,
– flow rate: 1 ml per minute,
– spectrophotometer set at 210 nm,
– injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to impurity A and azithromycin is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the eye drops.

Azithromycin Oral Suspension

Azithromycin Oral Suspension is a dry mixture of Azithromycin with buffering agents and other excipients, or is a homogeneous suspension in a suitable vehicle.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Azithromycin Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

Usual strength. 40 mg per ml.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

The contents of the sealed container comply with the following test.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.5 g.

Storage. Store protected from moisture.

The constituted suspension or the suspension complies with the tests stated under Oral liquids and with the following tests.

Identification

In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 7.5 to 11.0.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Prepare a 0.173 per cent w/v solution of *ammonium dihydrogen phosphate* with the pH adjusted to 10.0 with *strong ammonia solution*. Transfer 350 ml of the solution add 300 ml of *acetonitrile* and 350 ml of *methanol*. Mix well.

Test solution. Weigh a quantity of the oral suspension containing about 0.2 g of Azithromycin, dissolve in the solvent mixture, dilute to 50.0 ml with the solvent mixture and filter.

Reference solution (a). Dilute 1ml of the test solution to 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *6-demethyl-azithromycin IPRS (azithromycin impurity A IPRS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-cappped octadecylsilyl amorphous organosilica polymer for mass spectrometry (5µm) (Such as Waters Xterra),
- column temperature: 60°,
- mobile phase: A. a 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate* with the pH adjusted to 8.9 with *dilute orthophosphoric acid* or with *dilute sodium hydroxide solution*,
  B. a mixture of 25 volumes of *methanol* and 75 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

Name	Relative retention time	Correction factor
Azithromycin impurity L[1]	0.29	2.3
Azithromycin impurity M[2]	0.37	0.6
Azithromycin impurity E[3]	0.43	—
Azithromycin impurity F[4]	0.51	0.3
Azithromycin impurity D[5]	0.54	—
Azithromycin impurity J[6]	0.54	—
Azithromycin impurity I[7]	0.61	—
Azithromycin impurity C[8]	0.73	—
Azithromycin impurity N[9]	0.76	0.7
Azithromycin impurity H[10]	0.79	0.1
Azithromycin impurity A[11]	0.83	—
Azithromycin impurity P	0.92	—
Azithromycin (Retention time: about 45-50 minutes)	1.0	—
Azithromycin impurity O[12]	1.23	—
Azithromycin impurity G[13]	1.26	0.2
Azithromycin impurity B[14]	1.31	—

[1]azithromycin 3'-*N*-oxide,
[2]3'-(*N*,*N*-didemethyl)-3'-*N*-formylazithromycin,
[3]aminoazithromycin,

[4]3′-*N*-demethyl-3′-*N*- formylazithromycin,
[5]14-demethyl-14-(hydroxymethyl)azithromycin,
[6]13-*O*-decladinosylazithromycin,
[7]3′-*N*-demethylazithromycin,
[8]3″-*O*-demethylazithromycin,
[9]3′-de(dimethylamino)-3′-oxoazithromycin,
[10]3′-*N*-[[4-(acetylamino)phenyl]sulfonyl]-3′-*N*-demethylazithromycin,
[11]6-demethylazithromycin,
[12]2-desethyl-2-propylazithromycin,
[13]3′-*N*-demethyl-3′-*N*-[(4-methylphenyl)sulfonyl]azithromycin,
[14]3-deoxyazithromycin.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak eluting with an relative retention time of about 1.3 due to 3-deoxyazithromycin (azithromycin impurity B) is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). The sum of the areas of all the other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); ignore the peaks eluting before azithromycin impurity L and after azithromycin impurity B.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*.

Test solution. Weigh a quantity of the oral suspension containing about 0.1 g of Azithromycin, dissolve in the solvent mixture, dilute to 100.0 ml with the solvent mixture and filter.

Reference solution (a). A 0.1 per cent w/v solution of *azithromycin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *6-demethyl-azithromycin IPRS (azithromycin impurity A IPRS)* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with end capped polar embedded octadecylsilyl amorphous organosilica polymer (5µm) (Such as Waters Xterra),
- column temperature: 70°,
- mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of *dipotassium hydrogen phosphate*, previously adjusted to pH 6.5 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 100 µl.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromcyin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject reference solution (a) and the test solution.

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{38}H_{72}N_2O_{12}$, weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Azithromycin Tablets

Azithromycin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of azithromycin, $C_{38}H_{72}N_2O_{12}$.

Usual strengths. 250 mg; 500 mg.

Identification

In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of a buffer solution prepared by adding to 6 litres of *0.1 M dibasic sodium phosphate* about 40 ml of *hydrochloric acid*, adjusted to pH 6.0, adding 600 mg of *trypsin*, and mixing.
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

Test solution. Use the filtrate, dilute if necessary, with the mobile phase.

Reference solution. A solution of *azithromycin IPRS* in the dissolution medium suitably diluted with the mobile phase to obtain a solution having the same concentration as that of the test solution.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{38}H_{72}N_2O_{12}$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Prepare a 0.173 per cent w/v solution of *ammonium dihydrogen phosphate* with the pH adjusted to 10.0 with *strong ammonia solution*. Transfer 350 ml of the solution add 300 ml of *acetonitrile* and 350 ml of *methanol*. Mix well.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.2 g of Azithromycin, dissolve in the solvent mixture by shaking mechanically, dilute to 25.0 ml with the solvent mixture and filter.

Reference solution (a). A 0.008 per cent w/v solution of *azithromycin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *azithromycin impurity A IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-cappped octadecylsilyl amorphous organosilica polymer for mass spectrometry (5 µm) (Such as Waters Xterra),
- column temperature: 60°,
- mobile phase: A. a 0.18 per cent w/v solution of *anhydrous disodium hydrogen phosphate*, adjusted to pH 8.9 with *dilute orthophosphoric acid* or with *dilute sodium hydroxide solution*,
  B. a mixture of 25 volumes of *methanol* and 75 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

Name	Relative retention time	Correction factor
Azithromycin impurity L[1]	0.29	2.3
Azithromycin impurity M[2]	0.37	0.6
Azithromycin impurity E[3]	0.43	—
Azithromycin impurity F[4]	0.51	0.3
Azithromycin impurity D[5]	0.54	—
Azithromycin impurity J[6]	0.54	—
Azithromycin impurity I[7]	0.61	—
Azithromycin impurity C[8]	0.73	—
Azithromycin impurity N[9]	0.76	0.7
Azithromycin impurity H[10]	0.79	0.1
Azithromycin impurity A[11]	0.83	—
Azithromycin impurity P	0.92	—
Azithromycin (Retention time: about 45-50 minutes)	1.0	—
Azithromycin impurity O[12]	1.23	—
Azithromycin impurity G[13]	1.26	0.2
Azithromycin impurity B[14]	1.31	—

[1]azithromycin 3′-*N*-oxide,

[2]3′-(*N*,*N*-didemethyl)-3′-*N*-formylazithromycin,

[3]aminoazithromycin,

[4]3′-*N*-demethyl-3′-*N*- formylazithromycin,

[5]14-demethyl-14- (hydroxymethyl)azithromycin,

[6]13-*O*-decladinosylazithromycin,

[7]3′-*N*-demethylazithromycin,

[8]3″-*O*-demethylazithromycin,

[9]3′-de(dimethylamino)-3′-oxoazithromycin,

[10]3′-*N*-[[4-(acetylamino)phenyl]sulfonyl]-3′-*N*-demethylazithromycin,

[11]6-demethylazithromycin,

[12]2-desethyl-2-propylazithromycin,

[13]3′-*N*-demethyl-3′-*N*-[(4-methylphenyl)sulfonyl]azithromycin,

[14]3-deoxyazithromycin.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromycin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak eluting with an relative retention time of about 1.3 due to azithromycin impurity B is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). The sum of the areas of all the other secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with

an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); ignore the peaks eluting before azithromycin impurity L and after azithromycin impurity B.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of azithromycin, dissolve in the solvent mixture by shaking mechanically, dilute to 100 ml with the solvent mixture and filter.

Reference solution (a) . A 0.1 per cent w/v solution of *azithromycin IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of *azithromycin IPRS* and *azithromycin impurity A IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped polar embedded octadecylsilyl amorphous organosilica polymer (5 µm) (Such as Waters Xterra),
- column temperature: 70°,
- mobile phase: a mixture of 10 volumes of a 3.484 per cent w/v solution of *dipotassium hydrogen phosphate*, adjusted to pH 6.5 with *orthophosphoric acid*, 35 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 100 µl.

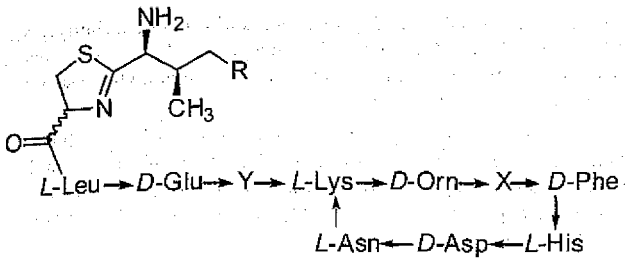
Inject reference solution (b). The chromatogram obtained shows peaks corresponding to azithromcyin and azithromycin impurity A. The test is not valid unless the resolution between these two peaks is at least 7.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the tablets.

B

Bacitracin



Name	Mol. Formula	X	Y	R
Bacitracin A	$C_{66}H_{103}N_{17}O_{16}S$	*L*-Ile	*L*-Ile	CH_3
Bacitracin B1	$C_{65}H_{101}N_{17}O_{16}S$	*L*-Ile	*L*-Ile	H
Bacitracin B2	$C_{65}H_{101}N_{17}O_{16}S$	*L*-Val	*L*-Ile	CH_3
Bacitracin B3	$C_{65}H_{101}N_{17}O_{16}S$	*L*-Ile	*L*-Val	CH_3

Bacitracin is a mixture of polypeptides produced by the growth of certain strains of *Bacillus licheniformis* or *Bacillus subtilis*. Its main components are Bacitracin A, B1, B2 and B3.

Bacitracin has a potency of not less than 60 Units of bacitracin activity per mg, calculated on the dried basis.

Category. Antibacterial (for topical use).

Description. A white or almost white, hygroscopic powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 14 volumes of *glacial acetic acid*, 57 volumes of *butanol* and 29 volumes of *water*.

Test solution. Dissolve 10 mg of the substance under examination in 0.34 per cent w/v solution of *hydrochloric acid* and dilute to 1.0 ml with the same solution.

Reference solution. Dissolve 10 mg of *bacitracin zinc IPRS* in 0.34 per cent w/v solution of *hydrochloric acid* and dilute to 1.0 ml with the same solution.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise above half of the plate. Dry the plate at 105°, spray with *ninhydrin solution* and heat at 110° for 5 minutes. The principal spot obtained with the test solution corresponds to spot similar in position, size and colour that in the chromatogram obtained with reference solution.

B. In the test for Composition, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with reference solution (a).

C. Ignite 0.2 g an insignificant residue remains which is not yellow at high temperature. Allow to cool. Dissolve the residue in 0.1 ml of *dilute hydrochloric acid*, 5 ml of *water* and 0.2 ml of strong *sodium hydroxide solution*. No white precipitate is formed.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1).

pH (2.4.24). 6.0 to 7.0, determined in solution A.

Composition. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Dissolve 4 g of *sodium edetate* in 100 ml of *water*, adjusted to pH 7.0 with *dilute sodium hydroxide solution*.

Test solution. Dissolve 0.1 g of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). Dissolve 20 mg of *bacitracin for system suitability IPRS* in 10.0 ml of the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (c). Heat about 4 ml of reference solution (a) in a water-bath for 30 minutes. Cool to room temperature (to generate impurities E, F, G and H *in situ*).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, end-capped, charged surface, ethylene-bridge octadecylsilane bonded to porous silica (3.5 µm),
- mobile phase: a mixture of 10 volumes of a buffer solution prepared by dissolving 27.2 g *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 6.0 with 3.48 per cent w/v solution of *dipotassium hydrogen phosphate*, 4.3 volumes of *acetonitrile*, 30 volumes of *water* and 55.7 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

Name	Relative retention time
Impurity A[1]	0.44
Impurity B[2]	0.52
Impurity C[3]	0.55
Bacitracin B1[4]	0.65
Bacitracin B2[5]	0.67

contd.

Name	Relative retention time
Bacitracin B3[6]	0.81
Impurity M[12]	0.87
Impurity N[13]	0.90
Impurity L[11]	0.93
Bacitracin A (retention time: about 20 minutes)	1.0
Impurity O[14]	1.2
Impurity P[15] and Q[16]	1.3
Impurity F[8]	1.6
Impurity G[9]	1.8
Impurity H[10]	2.1
Impurity E[7]	2.8

[1]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-valyl-D-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin D1, bacitracin C2),

[2]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-valyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin D2, bacitracin C3),

[3]4,10-anhydro[*N*-[[(4*R*)-2-[(1S,2S)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-valyl-*L*-lysyl-*D*-ornithyl-*L*-valyl-D-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin D3, bacitracin C1a),

[4]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin B1),

[5]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-valyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin B2),

[6]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-valyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin B3),

[7]4,10-anhydro[*N*-[[2-[(2*S*)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin F),

[8]4,10-anhydro[*N*-[[2-(2-methyl-1-oxopropyl)-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin H1)

[9]4,10-anhydro[*N*-[[2-[(2S)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-valyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin H2),

[10]4,10-anhydro[*N*-[[2-[(2S)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-valyl-L-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin H3),

[11]4,10-anhydro[*N*-[[(4*R*)-2-[(1*R*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamy-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin X),

[12]4,10-anhydro[*N*-[[2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin Y),

[13]4,10-anhydro[*N*-[[(4*S*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin Z),

[14]Mil = 5-methylene-*L*-isoleucine:4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-5-methylene-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin J1),

[15]Mil = 5-methylene-*L*-isoleucine:4,10-anhydro[*N*-[[(4*R*)-2-[(1S,2S)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-5-methylene-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin J2),

[16]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylpent-4-en-1-yl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin J3).

Inject reference solution (a). Identify the peaks due to impurity M, bacitracin A, bacitracin B1, bacitracin B2 and bacitracin B3.

Inject reference solution (a) and (b). The test is not valid unless the peak-to-valley ratio between the peaks due to bacitracin B1 and bacitracin B2 is not less than 1.2, where Hp is the height above the baseline of the peak due to bacitracin B2 and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B1 and between the peak due to impurity M and bacitracin B3 is not less than 1.1, where Hp is the height above the baseline of the peak due to impurity M and Hv is the height above the baseline of the lowest point of the curve separating from the peak due to bacitracin B3 in the chromatogram obtained with reference solution (a), the signal-to-noise ratio of the peak due to bacitracin A is not less than 50 in the chromatogram obtained with reference solution (b).

Inject the test solution. Run the chromatogram three times the retention time of the peak due to Bacitracin A. The area of the any peak corresponding to bacitracin A is not less than 45.0 per cent and the sum of the areas of the peaks due to bacitracin A, bacitracin B1, bacitracin B2 and bacitracin B3 is not less than 77 per cent. Ignore any peak with an area less than 0.25 per cent.

Related substances. Determine by liquid chromatography (2.4.14), as described under Composition with the following modifications.

Inject reference solution (a) and (c). Identify the peaks due to impurities A, B, C, L, M, N, O, P and Q in the chromatogram obtained with reference solution (a) and impurities E, F, G and H in the chromatogram obtained with reference solution (c).

Inject the test solution. The area of the any peak corresponding to impurity A is not more than 3.5 per cent, the area of the

peaks corresponding to impurities B and M, each of, is not more than 3.0 per cent, the area of any peak corresponding to impurity C is not more than 2.5 per cent, the area of any peak corresponding to impurity E is not more than 4.0 per cent, the sum of areas of the peaks corresponding to impurities O, P and Q is not more than 2.5 per cent, the sum of areas of the peaks corresponding to impurities F and G is not more than 2.0 per cent, the area of any peak corresponding to impurity H is not more than 1.0 per cent, the sum of areas of peaks corresponding to L and N is not more than 8.0 per cent and the area of any other secondary peak is not more than 2.0 per cent and the sum of areas of all the secondary peaks is not more than 23.0 per cent. Ignore any peak with an area less than 0.25 per cent.

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying under vacuum over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10). Use *bacitracin zinc IPRS* as the reference.

Bacitracin intended for use in the manufacture of Opthalmic Preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

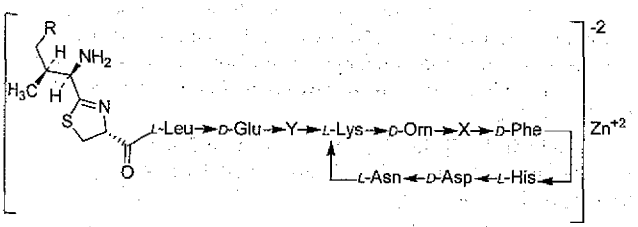
Bacitracin intended for use in the manufacture of Opthalmic Preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.8 Endotoxin Unit per mg of bacitracin.

Storage. Store protected from moisture, at a temperature between 2° to 8°. If the solution is sterile, the container is also sterile and temper-evident.

Labelling. The label states (1) the number of Units per mg; (2) whether or not the contents are intended for use in the manufacture of ophthalmic preparations.

Bacitracin Zinc



Name	Mol. Formula	X	Y	R
Bacitracin A	$C_{66}H_{103}N_{17}O_{16}S$	*L*-Ile	*L*-Ile	CH_3
Bacitracin B1	$C_{65}H_{101}N_{17}O_{16}S$	*L*-Ile	*L*-Ile	H
Bacitracin B2	$C_{65}H_{101}N_{17}O_{16}S$	*L*-Val	*L*-Ile	CH_3
Bacitracin B3	$C_{65}H_{101}N_{17}O_{16}S$	*L*-Ile	*L*-Val	CH_3

Bacitracin Zinc is zinc complex of bacitracin consists of a mixture of antimicrobial polypeptides produced by certain strains of *Bacillus licheniformis* or *Bacillus subtilis*. Its main components are Bacitracin A, B1, B2 and B3.

Bacitracin Zinc has a potency of not less than 60 Units of bacitracin activity per mg, calculated on the dried basis.

Category. Antibacterial (for topical use).

Description. A white or light yellowish-grey, hygroscopic powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 14 volumes of *glacial acetic acid*, 57 volumes of *butanol* and 29 volumes of *water*.

Test solution. Dissolve 10 mg of the substance under examination in 0.34 per cent w/v solution of *hydrochloric acid* and dilute to 1.0 ml with the same solution.

Reference solution. Dissolve 10 mg of *bacitracin zinc IPRS* in 0.34 per cent w/v solution of *hydrochloric acid* and dilute to 1.0 ml with the same solution.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise above half of the plate. Dry the plate at 105°, spray with *ninhydrin solution* and heat at 110° for 5 minutes. The principal spot obtained in the test solution corresponds to spot similar in position, size and colour that in the chromatogram obtained with the reference solution.

B. In the test for Composition, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with reference solution (a).

C. Ignite 0.15 g and allow to cool. Dissolve the residue in 1 ml of *dilute hydrochloric acid* and add 4 ml of *water*. The solution gives the reaction of zinc (2.3.1).

Tests

pH (2.4.24). 6.0 to 7.5, determined on the filtrate obtained by shaking 1.0 g for about 1 minutes with 10 ml of *carbon dioxide-free water* and filter.

Composition. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Dissolve 4 g of *disodium edetate* in 100 ml of *water*, adjusted to pH 7.0 with *dilute sodium hydroxide solution*.

Test solution. Dissolve 0.1 g of the substance under examination in 50.0 ml of the solvent mixture.

Reference solution (a). Dissolve 20 mg of *bacitracin for system suitability IPRS* in 10.0 ml of the solvent mixture.

Reference solution (b). Dilute 5.0 ml of the reference solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (c). Heat about 4 ml of reference solution (a) on a water-bath for 30 minutes. Cool to room temperature (to generate impurities E, F, G and H *in situ*).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with end-capped, charged surface, ethylene bridged octadecylsilane bonded to porous silica (3.5 µm),
- mobile phase: a mixture of 10 volumes of a buffer solution prepared by dissolving 27.2 g *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 6.0 with 3.5 per cent w/v solution of *dipotassium hydrogen phosphate*, 4.3 volumes of *acetonitrile*, 30 volumes of *water* and 55.7 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

Name	Relative retention time
Impurity A[1]	0.44
Impurity B[2]	0.52
Impurity C[3]	0.55
Bacitracin B1[4]	0.65
Bacitracin B2[5]	0.67
Bacitracin B3[6]	0.81
Impurity M[12]	0.87
Impurity N[13]	0.90
Impurity L[11]	0.93
Bacitracin A (retention time: about 20 minutes)	1.0
Impurity O[14]	1.2
Impurity P[15] and Q[16]	1.3
Impurity F[8]	1.6
Impurity G[9]	1.8
Impurity H[10]	2.1
Impurity E[7]	2.8

[1]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-D-α-glutamyl-*L*-isoleucyl-*L*-lysyl-D-ornithyl-*L*-valyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin D1, bacitracin C2),

[2]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-valyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin D2, bacitracin C3),

[3]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-valyl-*L*-lysyl-*D*-ornithyl-*L*-valyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin D3, bacitracin C1a),

[4]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin B1),

[5]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-valyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin B2),

[6]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-valyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin B3),

[7]4,10-anhydro[*N*-[[2-[(2*S*)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin F),

[8]4,10-anhydro[*N*-[[2-(2-methyl-1-oxopropyl)-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin H1)

[9]4,10-anhydro[*N*-[[2-[(2S)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-valyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin H2),

[10]4,10-anhydro[*N*-[[2-[(2S)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-valyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin H3),

[11]4,10-anhydro[*N*-[[(4*R*)-2-[(1*R*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamy-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin X),

[12]4,10-anhydro[*N*-[[2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin Y),

[13]4,10-anhydro[*N*-[[(4*S*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin Z),

[14]Mil = 5-methylene-*L*-isoleucine:4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-5-methylene-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin J1),

[15]Mil = 5-methylene-*L*-isoleucine:4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-5-methylene-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin J2),

[16]4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylpent-4-en-1-yl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-*L*-leucyl-*D*-α-glutamyl-*L*-isoleucyl-*L*-lysyl-*D*-ornithyl-*L*-isoleucyl-*D*-phenylalanyl-*L*-histidyl-*D*-α-aspartyl-*L*-asparagine] (bacitracin J3).

Inject reference solution (a). Identify the peaks due to impurity M, bacitracin A, bacitracin B1, bacitracin B2 and bacitracin B3.

Inject reference solution (a) and (b). The test is not valid unless the peak-to-valley ratio between the peaks due to bacitracin B1 and bacitracin B2 is not less than 1.2, where Hp is the height above the baseline of the peak due to bacitracin B2 and Hv is height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B1 and between the peaks due to impurity M and bacitracin B3 is not less than 1.1, where Hp is height above the baseline of the peak due to impurity M and Hv is height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B3 in the chromatogram obtained with reference solution (a). The signal-to-noise ratio of the peak due to bacitracin A is not less than 50 in the chromatogram obtained with reference solution (b).

Inject the test solution. Run the chromatogram three times the retention time of the peak due to Bacitracin A. The area of the any peak corresponding to Bacitracin A is not less than 45.0 per cent and the sum of area of the peaks due to bacitracin A, bacitracin B1, bacitracin B2 and bacitracin B3 is not less than 77 per cent. Ignore any peak with an area less than 0.25 per cent.

Related substances. Determine by liquid chromatography (2.4.14), as described under Composition with the following modifications.

Inject reference solution (a) and (c). Identify the peaks due to impurities A, B, C, L, M, N, O, P and Q in the chromatogram obtained with reference solution (a) and impurities E, F, G and H in the chromatogram obtained with reference solution (c).

Inject the test solution. The area of the any peak corresponding to impurity A is not more than 3.5 per cent, the area of the peaks corresponding to impurities B and M, each of, is not more than 3.0 per cent, the area of any peak corresponding to impurity C is not more than 2.5 per cent, the area of any peak corresponding to impurity E is not more than 4.0 per cent, the sum of areas of the peaks corresponding to impurities O, P and Q is not more than 2.5 per cent, the sum of areas of the peaks corresponding to impurities F and G is not more than 2.0 per cent, the area of any peak corresponding to impurity H is not more than 1.0 per cent, the sum of areas of peaks corresponding to L and N is not more than 8.0 per cent and the area of any other secondary peak is not more than 2.0 per cent and the sum of areas of all the secondary peaks is not more than 23.0 per cent. Ignore any peak with an area less than 0.25 per cent.

Zinc. 3.5 to 5.5 per cent, calculated on dried basis, determined on 0.2 g in a mixture of 2.5 ml of *dilute acetic acid* and 2.5 ml of *water*. Add 50 ml of *xylenol orange triturate* and sufficient *hexamethylenetetramine* to produce a red colour. Add 2 g of *hexamethylenetetramine* in excess. Titrate with *0.01 M disodium edetate* until a yellow colour is obtained.

1 ml of *0.01 M disodium edetate* is equivalent to 0.000654 g of Zn.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an vacuum over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

Assay. Weigh 50 mg, suspend in 5 ml of *water*, add 0.5 ml of *dilute hydrochloric acid* and dilute to 100.0 ml with *water*. Allow to stand at room temperature for 30 minutes. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the results in Units per mg.

Pyrogens (2.2.8). If intended for administration by spraying into internal body cavities without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1 ml of the supernatant liquid obtained by centrifuging a suspension containing 11mg per millilitre in a 0.9 per cent w/v solution of *sodium chloride*.

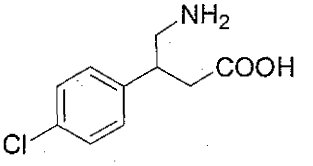
Bacitracin Zinc intended for administration as a spray in internal body cavities without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If the solution is sterile, the container is also sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) whether or not the contents are intended for administration as a spray in internal body cavities.

Baclofen



$C_{10}H_{12}ClNO_2$ Mol. Wt. 213.7

Baclofen is (*RS*)-4-amino-3-(4-chlorophenyl)butyric acid.

Baclofen contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{10}H_{12}ClNO_2$, calculated on the anhydrous basis.

Category. Muscle relaxant.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *baclofen IPRS or* with the refernce spectrum of baclofen.

B. When examined in the range 220 nm to 320 nm (2.4.7), a 0.07 per cent w/v solution shows three absorption maxima, at 259 nm, 266 nm and 275 nm. The specific absorbances at these maxima are 9.8 to 10.8, 11.5 to 12.7 and 8.4 to 9.3, respectively.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *anhydrous formic acid*, 5 volumes of *water*, 20 volumes of *methanol*, 30 volumes of *chloroform* and 40 volumes of *ethyl acetate*.

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *baclofen IPRS* in the mobile phase.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate and spray with *ninhydrin solution* until the plate is slightly wet. Place the plate in an oven maintained at 100° for 10 minutes. Examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. Dissolve 0.5 g in *1 M sodium hydroxide* and dilute to 25 ml with the same solvent. The freshly prepared solution is not more intensely coloured than reference solution BY5 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a). A 0.25 per cent w/v solution of *(4RS)-4-(4-chlorophenyl)pyrrolidin-2-one IPRS* (baclofen impurity A) in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Reference solution (c). Dilute 2 ml of the test solution to 100 ml with the mobile phase.

Reference solution (d). Dilute 2 ml of the test solution and 2 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a solution of 1.822 g of *sodium hexanesulphonate* in 1000 ml of a mixture of 56 volumes of *water*, 44 volumes of *methanol* and 0.5 ml of *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume: 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks corresponding to baclofen and impurity A is at least 2.0.

Inject reference solution (b), (c) and the test solution. Continue the chromatography for 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak corresponding to baclofen impurity A is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Assay. Weigh 0.15 g and dissolve in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02137 g of $C_{10}H_{12}ClNO_2$.

Storage. Store protected from moisture.

Baclofen Oral Solution

Baclofen Oral Solution is a solution of Baclofen in a suitable aqueous vehicle.

Baclofen Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of baclofen, $C_{10}H_{12}ClNO_2$.

Usual strength. 1 mg per ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 35 volumes of *acetonitrile* and 65 volumes of *water*.

Mobile phase. A mixture of 20 volumes of *glacial acetic acid*, 20 volumes of *water* and 80 volumes of *butan-1-ol*.

Test solution. Dilute a volume of the oral solution containing 5 mg of Baclofen to 100 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *baclofen IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air. Place an evaporating dish containing a mixture of 4 ml of *water*, 1 ml of *7 M hydrochloric acid* and 0.5 g of *potassium permanganate* in a chromatography tank, close the tank and allow to stand for 2 minutes. Place the plate in the tank, close the tank and leave the plate in contact with the vapour for 1 minute. After removal of the plate, place it in a current of cold air until an area of coating below the line of application shows only a faint blue colour on the addition of 0.05 ml of *potassium iodide and starch solution*. Spray the plate with *potassium iodide and starch solution* and examine in daylight. The chromatogram obtained with the test solution exhibits a spot that corresponds to the spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Lactam. Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

Test solution. Use the test solution prepared for the Assay.

Reference solution (a). A solution containing 0.0002 per cent w/v of *(4RS)-4-(4-chlorophenyl)pyrrolidin-2-one IPRS (baclofen impurity A)* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v of *baclofen IPRS*, 0.0003 per cent w/v of *propyl 4-hydroxybenzoate*, 0.0003 per cent w/v of *methyl 4-hydroxybenzoate* and 0.0002 per cent w/v of *baclofen impurity A* in the mobile phase.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to methyl-4-hydroxybenzoate and baclofen impurity A and between the peaks due to baclofen impurity A and propyl-4-hydroxybenzoate is not less than 5.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to baclofen impurity A (lactam) is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (2.0 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14):

Test solution. Dilute a weighed quantity of the oral solution containing about 5 mg of Baclofen to 50.0 ml with the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *baclofen IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v of *baclofen IPRS*, 0.0003 per cent w/v of *propyl 4-hydroxybenzoate* and 0.0002 per cent w/v of *baclofen impurity A IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Nucleosil C18),
- mobile phase: a solution prepared by dissolving 5 g of *sodium dodecyl sulphate* in a mixture of 5 ml of *orthophosphoric acid* and 650 ml of *water* and diluting to 1000 ml with *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to baclofen impurity A and propyl-4-hydroxybenzoate is not less than 5.0 and the relative standard deviation for replicate injections for baclofen peak is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_{10}H_{12}ClNO_2$, weight in volume.

Storage. Store protected from light, at a temperature not exceeding 30°. Do not freeze.

Baclofen Tablets

Baclofen tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of baclofen, $C_{10}H_{12}ClNO_2$.

Usual strengths. 10 mg; 20 mg.

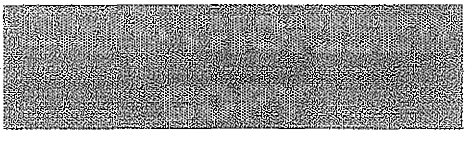
Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 4 volumes of *ethanol* and 1 volume of *glacial acetic acid*.

Mobile phase. A mixture of 80 volumes of *butan-1-ol*, 20 volumes of *glacial acetic acid* and 20 volumes of *water*.

Test solution. Disperse a quantity of the powdered tablets containing 20 mg of Baclofen with 20 ml of the solvent mixture for 30 minutes and filter.



Reference solution. A 0.1 per cent w/v solution of *baclofen IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 100° for 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Lactam. Determine by liquid chromatography (2.4.14).

Test solution. Mix with the aid of ultrasound a quantity of the powdered tablets containing 0.1 g of Baclofen with 50 ml of the mobile phase for 30 minutes, shaking occasionally to disperse the sample, and filter through a glass-fibre filter (such as Whatman GF/C).

Reference solution (a). A solution containing 0.004 per cent w/v of *(4RS)-4-(4-chlorophenyl)pyrrolidin-2-one IPRS (baclofen impurity A)* in the mobile phase.

Reference solution (b). A solution containing 0.2 per cent w/v of *baclofen IPRS* and 0.004 per cent w/v of *(4RS)-4-(4-chlorophenyl)pyrrolidin-2-one IPRS* (baclofen impurity A) in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 5 volumes of *glacial acetic acid*, 44 volumes of *methanol* and 50 volumes of water containing 1.822 g per litre of *sodium hexanesulphonate*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to baclofen and baclofen impurity A is at least 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to baclofen impurity A (lactam) is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (2.0 per cent).

Dissolution (2.5.2)

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

Test solution. Use the filtrate as given above.

Reference solution. A 0.001 per cent w/v solution of *baclofen IPRS* in the dissolution medium.

Calculate the content of $C_{10}H_{12}ClNO_2$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{10}H_{12}ClNO_2$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Add a quantity of whole tablets containing 0.1 g of Baclofen to 25 ml of a mixture of 100 volumes of *water* and 1 volume of *glacial acetic acid* and disperse with the aid of ultrasound. Dilute to 50.0 ml with *methanol*, filter and use the filtrate.

Reference solution. A 0.2 per cent w/v solution of *baclofen IPRS* in a mixture of 100 volumes of *methanol*, 100 volumes of *water* and 1 volume of *glacial acetic acid*.

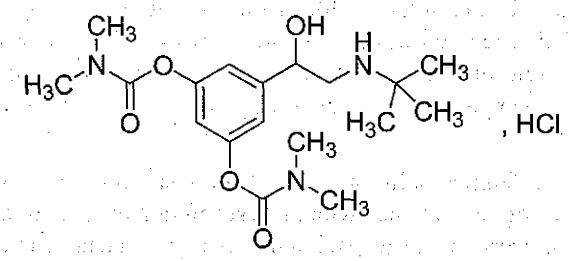
Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilyl silica gel (10 µm) (such as Nucleosil C18),
- mobile phase: *0.01 M sodium hexanesulphonate* in a mixture of 100 volumes of *methanol*, 100 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{12}ClNO_2$, in the tablets.

Bambuterol Hydrochloride



$C_{18}H_{29}N_3O_5,HCl$ Mol Wt. 403.9

Bambuterol Hydrochloride is 5-[(1*RS*)-2-[(1, 1-dimethylethyl) amino]-1-hydroxyethyl]-1,3-phenylene bis(dimethyl-carbamate) hydrochloride.

Bambuterol Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of the $C_{18}H_{29}N_3O_5$,HCl calculated on the anhydrous basis.

Category. Bronchodilator.

Description. A white or almost white, crystalline powder .It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bambuterol hydrochloride IPRS* or with the reference spectrum of bambuterol hydrochloride. If the sample spectrum shows differences in the position of the absorption maximum as compared to reference spectrum, treat the sample and reference substance as follows. Dissolve the substance under examination in a mixture of 1 volume of *water* and 6 volumes of *acetone*, cool in ice to precipitate and dry precipitate in vacuum at 50°. On the residue, determine by infrared absorption spectrophotometry (2.4.6) and compare the spectra.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

Acidity or alkalinity. To 10 ml of 20 per cent w/v solution in *carbon dioxide-free water*, add 0.2 ml of *methyl red solution* and 0.2 ml of *0.01 M hydrochloric acid*; the solution is red. Add 0.4 ml of *0.01 M sodium hydroxide*; the solution is yellow.

Optical rotation (2.4.22). – 0.10° to + 0.10°, determined on 2.0 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a) A 0.01 per cent w/v solution of *formoterol fumarate dihydrate IPRS* in the mobile phase. Mix 0.8 ml of the solution with 0.4 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Reference solution (b) Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with base-deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 1.3 g of *sodium octanesulphonate* in 430 ml of a mixture of 25 volumes of *acetonitrile* and 75 volumes of *methanol* and 570 ml of a buffer solution prepared by dissolving 6.9 g of *sodium dihydrogen phosphate monohydrate* in *water* and dilute to 1000 ml with *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks corresponding to bambuterol and formoterol is not less than 5.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.5 g.

Assay. Dissolve 0.32 g in 50 ml of *ethanol (95 per cent)*, add 5 ml of *0.01 M hydrochloric acid* and titrate with *0.1 M sodium hydroxide*, determining the end- point potentiometrically (2.4.25). Read the volume added between the two points of inflection.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.04039 g of $C_{18}H_{29}N_3O_5$,HCl.

Bambuterol Tablets

Bambuterol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bambuterol hydrochloride, $C_{18}H_{29}N_3O_5$,HCl.

Usual strengths. 10 mg; 20 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *bambuterol hydrochloride IPRS* in the mobile phase and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{29}N_3O_5$,HCl in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{18}H_{29}N_3O_5$,HCl.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse a quantity of powdered tablets containing 50 mg of Bambuterol Hydrochloride in 20 ml of the mobile phase, with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the mobile phase, filter. Dilute 5.0 ml of the solution to 10.0 ml with the mobile phase.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of the areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization method.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay.

Test solution. Disperse one tablet in 50 ml of the mobile phase with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with the mobile phase.

Reference solution. Prepare a solution using *bambuterol hydrochloride IPRS* in the mobile phase to obtain the same concentration as expected in the test solution.

Calculate the content of $C_{18}H_{29}N_3O_5$.HCl in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powdered tablets containing 50 mg of Bambuterol Hydrochloride in 20 ml of the mobile phase, with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the mobile phase, filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *bambuterol hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 1.3 g of *sodium octane sulphonate* in 430 ml of a mixture of 25 volumes of *acetonitrile* with 75 volumes of *methanol* and 570 ml of *0.05 M phosphate buffer pH 3.0*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{29}N_3O_5$,HCl in the tablets.

Barium Sulphate

$BaSO_4$ Mol. Wt. 233.4

Category. Diagnostic aid (radio-opaque medium for gastrointestinal tract).

Description. A fine, heavy, white powder, free from gritty particles; odourless.

Identification

A. Boil 0.2 g with 5 ml of a 50 per cent w/v solution of *sodium carbonate* for 5 minutes, add 10 ml of *water* and filter. Reserve the residue for test B. Acidify the filtrate with *dilute hydrochloric acid*; the solution gives the reactions of sulphates (2.3.1).

B. Wash the residue obtained in test A three times with successive small quantities of *water*. To the residue add 5 ml of *dilute hydrochloric acid*, filter and add to the filtrate 0.3 ml of *dilute sulphuric acid*; a white precipitate is formed which is insoluble in *dilute sodium hydroxide solution*.

Tests

Acidity or alkalinity. Heat 5.0 g with 20 ml of *carbon dioxide-free water* on a water-bath for 5 minutes and filter. To 10 ml of the filtrate add 1 drop of *bromothymol blue solution*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the solution.

Arsenic (2.3.10). Disperse 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Boil 4.0 g with a mixture of 2 ml of *glacial acetic acid* and 48 ml of *water* for 10 minutes. Add *water* to

make upto 50 ml, filter and reject the first 5 ml of the filtrate. 25 ml of the filtrate complies with the limit test for heavy metals, Method A (10 ppm).

Loss on ignition (2.4.20). Not more than 2.5 per cent, determined on 1.0 g at 600°.

Phosphate. Boil 1 g with a mixture of 3 ml of *nitric acid* and 5 ml of *water* for 5 minutes and add *water* to restore the original volume. Filter through a filter paper previously washed with *dilute nitric acid*. Add to the warm filtrate an equal volume of *ammonium molybdate solution*; no yellow precipitate is formed.

Sulphide. Boil 10 g with a mixture of 10 ml of *dilute hydrochloric acid* and 90 ml of *water* for 10 minutes. Expose a *lead acetate paper* to the vapours; the paper does not darken.

Acid-soluble substances. Cool the mixture obtained in the test for Sulphide, add *water* to restore the original volume and filter through a filter paper previously washed with a mixture of 10 ml of *dilute hydrochloric acid* and 90 ml of *water*, returning the first portions, if necessary, to obtain a clear filtrate. Evaporate 50 ml of the filtrate to dryness on a water-bath and add 2 drops of *hydrochloric acid* and 10 ml of hot *water*. Filter again through acid-washed paper, prepared as directed above, wash the filter paper with 10 ml of hot *water* and evaporate the combined filtrate and washings. Dry the residue at 105°, cool and weigh (0.3 per cent).

Soluble barium salts. Digest the residue obtained in the test for Acid-soluble substances with 10 ml of *water* and filter through a filter paper previously washed with a mixture of 10 ml of *dilute hydrochloric acid* and 90 ml of *water*. Add 0.5 ml of *dilute sulphuric acid* to the clear filtrate and set aside for 30 minutes; no turbidity is produced.

Barium Sulphate Oral Suspension

Barium Sulphate Oral Suspension is a suspension of Barium Sulphate in a suitable aqueous vehicle.

Barium Sulphate Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of barium sulphate, $BaSO_4$.

Description. A smooth, white or creamy white suspension.

Identification

Evaporate 1 ml to dryness and ignite to constant weight. The residue complies with the following tests.

A. To 0.2 g, add 5 ml of a 50 per cent w/v solution of *sodium carbonate* and boil for 5 minutes, add 10 ml of *water* and filter. Reserve the residue for test B. Acidify the filtrate with *2 M hydrochloric acid*. The solution gives the reactions of sulphates (2.3.1).

B. Wash the residue reserved in test A with *water*, add 5 ml of *2 M hydrochloric acid*, mix well and filter. Add 0.3 ml of *1 M sulphuric acid* to the filtrate. A white precipitate is produced which is insoluble in *2 M hydrochloric acid*.

Tests

pH (2.4.24). 3.5 to 8.5.

Microbial contamination (2.2.9). The total aerobic viable count is not more than 100 cfu per ml, the total combined molds and yeasts count is not more than 10 cfu per ml. 1 g is free from *staphylococcus aureus*, and *pseudomonas aeruginosa* and 10 g is free from *salmonella* species.

Other tests. Comply with the tests stated under Oral liquids.

Assay. Evaporate to dryness a quantity containing 0.6 g of Barium Sulphate in a platinum dish on a water-bath and add 5 g of *sodium carbonate* and 5 g of *potassium carbonate sesquihydrate* and mix. Heat to 100° and maintain at this temperature for 15 minutes. Allow to cool and suspend the residue in 150 ml of *water*. Wash the crucible with 2 ml of *6 M acetic acid* and add the washings to the suspension. Cool in ice and filter by decantation, transferring as little of the solid matter as possible to the filter. Wash the residue with successive quantities of a 2 per cent w/v solution of *sodium carbonate* until the washings are free from sulphate and discard the washings. Add 5 ml of *2M hydrochloric acid* to the filter and wash through into the vessel containing the bulk of the solid matter with *water*. Add 5 ml of *hydrochloric acid* and dilute to 100 ml with *water*. Add 10 ml of a 40 per cent w/v solution of *ammonium acetate*, 25 ml of a 10 per cent w/v solution of *potassium dichromate* and 10 g of *urea*. Cover, digest in an oven at 80° to 85° for 16 hours and filter whilst still hot through a sintered-glass filter (porosity No. 4), washing the precipitate initially with a 0.5 per cent w/v solution of *potassium dichromate* and finally with 2 ml of *water*. Dry to constant weight at 105°.

1 g of the residue is equivalent to 0.9213 g of $BaSO_4$.

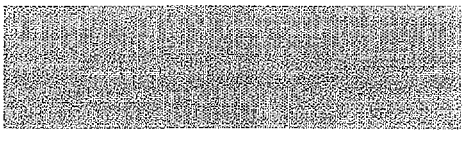
Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $BaSO_4$, weight in volume.

Barium Sulphate for Suspension

Barium Sulphate for Suspension is a dry mixture of Barium Sulphate with suitable dispersing agents and may contain suitable flavours and suitable antimicrobial preservatives.

Barium Sulphate for Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of barium sulphate, $BaSO_4$.

Description. A fine, white or creamy white powder.



Identification

A. Ignite 1 g to constant weight. To 0.2 g of the residue, add 5 ml of a 50 per cent w/v solution of *sodium carbonate* and boil for 5 minutes, add 10 ml of *water* and filter. Reserve the residue for test B. Acidify the filtrate with *2 M hydrochloric acid*; the solution gives the reactions of sulphates (2.3.1).

B. Wash the residue obtained in test A with *water*, add 5 ml of *2 M hydrochloric acid*, filter. Add to the filtrate, 0.3 ml of *1 M sulphuric acid*; a white precipitate is produced which is insoluble in *2 M hydrochloric acid*.

Tests

pH (2.4.24). 3.5 to 8.5, determined in a suspension containing 60 per cent w/v Barium Sulphate in *water*.

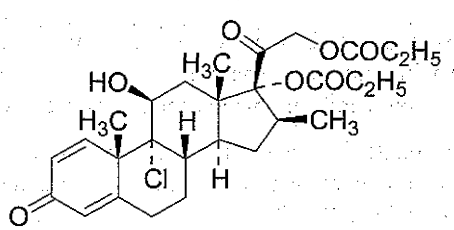
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. To a quantity containing 0.6 g of Barium Sulphate in a platinum crucible, add 5 g of *sodium carbonate* and 5 g of *potassium carbonate sesquihydrate* and mix. Heat to 100° and maintain at this temperature for 15 minutes. Allow to cool and suspend the residue in 150 ml of *water*. Wash the crucible with 2 ml of *6 M acetic acid* and add the washings to the suspension. Cool in ice and filter by decantation, transferring as little of the solid matter as possible to the filter. Wash the residue with successive quantities of a 2 per cent w/v solution of *sodium carbonate* until the washings are free from sulphate and discard the washings. Add 5 ml of *2 M hydrochloric acid* to the filter and wash through into the vessel containing the bulk of the solid matter with *water*. Add 5 ml of *hydrochloric acid* and dilute to 100 ml with *water*. Add 10 ml of a 40 per cent w/v solution of *ammonium acetate*, 25 ml of a 10 per cent w/v solution of *potassium dichromate* and 10 g of *urea*. Cover, digest in an oven at 80° to 85° for 16 hours and filter whilst still hot through a sintered-glass filter (porosity No. 4), washing the precipitate initially with a 0.5 per cent w/v solution of *potassium dichromate* and finally with 2 ml of *water*. Dry to constant weight at 105°.

1 g of the residue is equivalent to 0.9213 g of $BaSO_4$.

Beclomethasone Dipropionate



$C_{28}H_{37}ClO_7$ Mol. Wt. 521.1

Beclomethasone Dipropionate is 9α-chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17,21-diyl dipropionate.

Beclomethasone Dipropionate is anhydrous or contains one molecule of water of hydration.

Beclomethasone Dipropionate contains not less than 96.0 per cent and not more than 103.0 per cent of $C_{28}H_{37}ClO_7$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white to creamy-white, crystalline powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *beclomethasone dipropionate IPRS* or with the reference spectrum of beclomethasone dipropionate.

B. Determine by the oxygen flask method (2.3.34), on 25 mg and use a mixture of 20 ml of *water* and 1 ml of *1 M sodium hydroxide* as the absorbing liquid. The liquid gives reaction (A) of chlorides (2.3.1).

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). + 88.0° to + 94.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption. Dissolve 50.0 mg in sufficient *ethanol (95 per cent)* to produce 100.0 ml and dilute 2.0 ml of the solution to 50.0 ml with the same solvent. Absorbance of the resulting solution at the maximum at about 238 nm, 0.57 to 0.60 (2.4.7).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent for anhydrous form; 2.8 per cent to 3.8 percent for monohydrate form, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 70 mg of the substance under examination, dissolve in *methanol* and dilute to 50.0 ml with same solvent. To 4.0 ml of the solution add 4.0 ml of a 0.12 per cent w/v solution of *testosterone propionate IPRS* (internal standard) in *methanol*.

Reference solution. Dissolve a weighed quantity of *beclomethasone dipropionate IPRS* in *methanol* and dilute to obtain a solution having a known concentration of about 1.4 mg per ml. To 4.0 ml of the solution add 4.0 ml of a 0.12 per

cent w/v solution of *testosterone propionate IPRS* (internal standard) in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 3 volumes of *acetonitrile* and 2 volumes of *water*, or such that the retention time of beclomethasone dipropionate is approximately 6 minutes and that of testsosterone propionate is approximately 10 minutes,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{28}H_{37}ClO_7$.

Storage. Store protected from light.

Beclomethasone Inhalation

Beclomethasone Dipropionate Inhalation;
Beclomethasone Inhalation Aerosol

Beclomethasone Inhalation is a suspension of Beclomethasone Dipropionate in a suitable liquid in a suitable pressurised container.

Beclomethasone Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount per inhalation of beclomethasone dipropionate, $C_{28}H_{37}ClO_7$, by actuation of the valve.

Identification

A. Discharge the container a sufficient number of times at low relative humidity into a mortar to obtain about 2 mg of anhydrous Beclomethasone Dipropionate. Heat at 110° for 2 hours at a pressure of 2kPa, cool, grind the residue thoroughly with 0.1 g of potassium bromide, add a further 0.2 g of potassium bromide and mix thoroughly.

On the resultant dispersion determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *beclomethasone dipropionate IPRS* or with the reference spectrum of beclomethasone dipropionate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to beclomethasone dipropionate in the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 3 volumes of *methanol* and 97 volumes of *dichloroethane*.

Test solution. Discharge from the container into a small, dry flask a sufficient number of times to obtain 0.5 mg of Beclomethasone Dipropionate and dissolve the residue in 2 ml of *acetone*. Evaporate the solution to a volume such that the whole solution can be applied to the plate.

Reference solution (a). A 0.1 per cent w/v solution of *beclomethasone dipropionate IPRS* in *acetone*.

Reference solution (b). Dilute 5 ml of reference solution (a) to 10 ml with *acetone*.

Reference solution (c). Dilute 5 ml of reference solution (a) to 20 ml with *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *alkaline tetrazolium blue solution* and heat at 50° for 5 minutes. Cool and spray again with *alkaline tetrazolium blue solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a), not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent). Ignore any spot with an R_f value of more than 0.85.

Other tests. Comply with the tests stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Follow the procedure described under Assay wherever the amount of active substance is to be determined in any test.

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Use 40 ml of *dehydrated methanol* as the solvent. Discharge the number of deliveries that constitute the minimum recommended dose, keep the solution on a water-bath for 5 minutes to expel the propellants. Transfer the solution and washings to a flask containing sufficient *testosterone propionate IPRS* (internal standard) in *methanol* that, on dilution to a suitable volume with appropriate amounts of *water* and *methanol*, the final solution contains 0.00015 per cent w/v each of testosterone propionate and beclomethasone dipropionate in the methanol-water mixture in the proportions 70:30 by volume.

Determine by liquid chromatography (2.4.14).

Test solution. The diluted solution obtained as given above.

Reference solution. A solution containing 0.00015 per cent w/v each of the internal standard and *beclomethasone dipropionate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *water*, adjusted if necessary so that the resolution between the peaks due to beclomethasone dipropionate and the internal standard is not less than 2.0,
- flow rate: 2 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is not less than 2.0.

Inject the reference solution and the test solution.

Calculate the amount of $C_{28}H_{37}ClO_7$ delivered per actuation of the valve.

Determine the content of active ingredient for second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of $C_{28}H_{37}ClO_7$ delivered per actuation of the valve meets the requirements.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the amount of active ingredient delivered per inhalation.

White Beeswax

White Beeswax is obtained by bleaching Yellow Beeswax.

Category. Pharmaceutical aid.

Description. Yellowish-white pieces or plates, translucent when thin, with a fine-grained, matt, non-crystalline fracture; becomes soft and pliable when warmed by hand.

Tests

Melting range (2.4.21). 61° to 65°, determined by Method IV.

Acid value (2.3.23). 5 to 15, determined by the following method. Weigh 5.0 g in a 250-ml conical flask fitted with a reflux condenser, add 40 ml of *xylene* and a few glass beads, heat until dissolved, add 20 ml of *ethanol (95 per cent)* and 0.5 ml of *phenolphthalein solution* and titrate the hot solution with *0.5 M ethanolic potassium hydroxide* until a red colour persists for at least 10 seconds (n_1 ml). Repeat the procedure omitting the substance under examination (n_2 ml). Calculate the Acid value from the expression $28.05(n_1 - n_2)/w$, where w is the weight, in g, of the substance taken.

Ester value (2.3.26). 75 to 95, determined by subtracting the Acid value from the Saponification value.

Ratio number. The Ester value divided by the Acid value is between 5 and 19.

Saponification value (2.3.37). 87 to 104, determined by the following method. Weigh 2.0 g, add 30 ml of a mixture of equal volumes of *xylene* and *ethanol (95 per cent)* and a few glass beads, heat until dissolved, add 25.0 ml of *0.5 M ethanolic potassium hydroxide* and heat under a reflux condenser for 3 hours. Titrate the hot solution immediately with *0.5 M hydrochloric acid* using 1 ml of *phenolphthalein solution* as indicator, bringing the solution back to boil several times during the titration (n_1 ml). Repeat the procedure omitting the substance under examination (n_2 ml). Calculate the Saponification value from the expression $28.05(n_2 - n_1)/w$, where w is the weight, in g, of the substance taken.

Fats, fatty acids, Japan wax and resin. Boil 5.0 g for 10 minutes with 80 ml of a 10 per cent w/v solution of *sodium hydroxide*, replace the water lost by evaporation, cool, filter the solution through a plug of glass wool and acidify with *hydrochloric acid*; no precipitate is produced.

Ceresin, paraffin and other waxes. To 3.0 g in a 100-ml round-bottomed flask add 30 ml of a 4 per cent w/v solution of *potassium hydroxide* in *aldehyde-free ethanol (95 per cent)* and boil gently under a reflux condenser for 2 hours. Remove the condenser and immediately insert a thermometer, place the flask in a water-bath at 80° and allow to cool with continuous swirling. The solution may be opalescent, but no precipitate is formed before the temperature reaches 65°.

Glycerin and other polyhydric alcohols. To 0.2 g add 10 ml of *ethanolic potassium hydroxide solution*, heat under a reflux condenser in a water-bath for 30 minutes, add 50 ml of *1 M sulphuric acid*, cool and filter. Rinse the flask and filter with *1 M sulphuric acid*, combine the filtrate and washings and dilute to 100 ml with *1 M sulphuric acid* (solution A). Into two matched test-tubes introduce, respectively, 1 ml of solution A and 1 ml of a 0.001 per cent w/v solution of *glycerin* in *1 M sulphuric acid* (solution B). Add 0.5 ml of a 1.07 per cent w/v solution of *sodium periodate* to each tube, mix, allow to stand for 5 minutes, add to each tube 1 ml of *decolorised fuchsin solution* and mix; any precipitate disappears. Place the tubes in a beaker containing water at 40° and observe for 10 to 15 minutes during cooling. Any bluish violet colour in the tube containing solution A is not more intense than that in the tube containing solution B (0.5 per cent w/w, calculated as glycerin).

Yellow Beeswax

Yellow beeswax is the wax obtained by melting the walls of the honeycomb of the bee, *Apis mellifera* Linn. with hot water and removing the foreign matter.

Category. Pharmaceutical aid.

Description. Yellow or light brown pieces or plates, with a fine-grained, matt, non-crystalline fracture; becomes soft and pliable when warmed by hand. It is tasteless and does not stick to the teeth.

Tests

Melting range (2.4.21). 61° to 65°, determined by Method IV.

Acid value (2.3.23). 5 to 15, determined by the following method. Weigh 5.0 g in a 250-ml conical flask fitted with a reflux condenser, add 40 ml of *xylene* and a few glass beads, heat until dissolved, add 20 ml of *ethanol (95 per cent)* and 0.5 ml of *phenolphthalein solution* and titrate the hot solution with *0.5 M ethanolic potassium hydroxide* until a red colour persists for at least 10 seconds (n_1 ml). Repeat the procedure omitting the substance under examination (n_2 ml). Calculate the Acid value from the expression:

$$= \frac{28.05(n_1 - n_2)}{w}$$

where w is the weight, in g, of the substance taken.

Ester value (2.3.26). 75 to 95, determined by subtracting the Acid value from the Saponification value.

Ratio number. The Ester value divided by the Acid value is between 5 and 19.

Saponification value (2.3.37). 87 to 104, determined by the following method. Weigh 2.0 g, add 30 ml of a mixture of equal volumes of *xylene* and *ethanol (95 per cent)* and a few glass beads, heat until dissolved, add 25.0 ml of *0.5 M ethanolic potassium hydroxide* and heat under a reflux condenser for 3 hours. Titrate the hot solution immediately with *0.5 M hydrochloric acid* using 1 ml of *phenolphthalein solution* as indicator, bringing the solution back to boil several times during the titration (n_1 ml). Repeat the procedure omitting the substance under examination (n_2 ml). Calculate the Saponification value from the expression:

$$= \frac{28.05(n_2 - n_1)}{w}$$

where w is the weight, in g, of the substance taken.

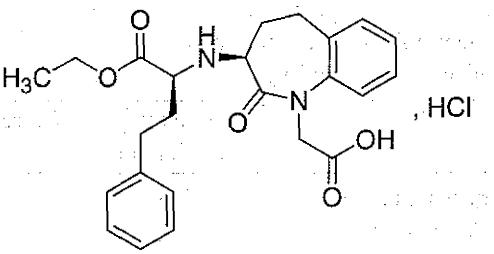
Fats, fatty acids, Japan wax and resin. Boil 5 g for 10 minutes with 80 ml of a 10 per cent w/v solution of *sodium hydroxide*, replace the water lost by evaporation, cool, filter the solution through a plug of glass wool and acidify with *hydrochloric acid*; no precipitate is produced.

Ceresin, paraffin and other waxes. To 3.0 g in a 100-ml round-bottomed flask add 30 ml of a 4 per cent w/v solution of *potassium hydroxide* in *aldehyde-free ethanol (95 per cent)* and boil gently under a reflux condenser for 2 hours. Remove the condenser and immediately insert a thermometer, place the flask in a water-bath at 80° and allow to cool with continuous swirling. The solution may be opalescent, but no precipitate is formed before the temperature reaches 65°.

Glycerin and other polyhydric alcohols. To 0.2 g add 10 ml of *ethanolic potassium hydroxide solution*, heat under a reflux condenser in a water-bath for 30 minutes, add 50 ml of *1 M sulphuric acid*, cool and filter. Rinse the flask and filter with *1 M sulphuric acid*, combine the filtrate and washings and dilute to 100 ml with *1 M sulphuric acid* (solution A). Into two matched test-tubes introduce, respectively, 1 ml of solution A and 1 ml of a 0.001 per cent w/v solution of *glycerin* in *1 M sulphuric acid* (solution B). Add 0.5 ml of a 1.07 per cent w/v solution of *sodium periodate* to each tube, mix, allow to stand for 5 minutes, add to each tube 1 ml of *decolorised fuchsin solution* and mix; any precipitate disappears. Place the tubes in a beaker containing water at 40° and observe for 10 to 15 minutes during cooling. Any bluish violet colour in the tube containing solution A is not more intense than that in the tube containing solution B (0.5 per cent w/w, calculated as glycerin).

Storage. Store in well-closed containers.

Benazepril Hydrochloride



$C_{24}H_{28}N_2O_5$, HCl Mol. Wt. 461.0

Benazepril Hydrochloride is {(3*S*)-3-[(1*S*)-1-Ethoxycarbonyl-3-phenylpropylamino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepin-1-yl}acetic acid hydrochloride.

Benazepril Hydrochloride contains not less than 97.5 per cent and not more than 102.0 per cent of $C_{24}H_{28}N_2O_5$, HCl, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or almost white, crystalline powder, hygroscopic. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benazepril hydrochloride IPRS* or with the reference spectrum of benazepril hydrochloride.

B. Specific optical rotation (2.4.22). – 141.0° to – 136.0°, determined in a 2.0 per cent w/v solution in *ethanol*.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Test solution (b). Dilute 10.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *benazepril hydrochloride IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a buffer solution prepared by adding 0.2 ml of *glacial acetic acid* and 0.81 g of *tetrabutylammonium bromide* to 1000 ml in a mixture of 360 volumes of *water* and 640 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 25 µl.

Name	Relative retention time	Correction factor
Benazepril impurity E[1]	0.3	0.5
Benazepril impurity F[2]	0.4	0.7
Benazepril impurity C[3]	0.5	---
Benazepril (Retention time: about 6 minutes)	1.0	---
Benazepril impurity B[4]	1.8	—
Benazepril impurity D[5]	2.0	—
Benazepril impurity G[6]	2.5	—

[1][(3*S*)-3-amino-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl]acetic acid,

[2]1,1-dimethylethyl [(3*S*)-3-amino-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl]acetate

[3](2*S*)-2-[[(3*S*)-1-(carboxymethyl)-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-3-yl]amino]-4-phenylbutanoic acid,

[4][(3*RS*)-3-[[(1*SR*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2-oxo-2,3,4,5- tetrahydro-1*H*-1-benzazepin-1-yl]acetic acid,

[5][(3*S*)-3-[[(1*S*)-3-cyclohexyl-1-(ethoxycarbonyl)propyl]amino]-2-oxo-2,3,4,5- tetrahydro-1*H*-1-benzazepin-1-yl]acetic acid,

[6]ethyl(2*S*)-2-[[(3*S*)-1-(2-ethoxy-2-oxoethyl)-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-3-yl]amino]-4-phenylbutanoate.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and tailing factor is not more than 2.0.

Inject reference solution (b) and test solution (a). Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with test solution (a) the area of any peak corresponding to benazepril impurity B is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak corresponding to benazepril impurity C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any peaks corresponding to benazepril impurities D, E, F and G is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), area of any other peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with the area less than 0.25 times area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dissolve 5 mg of *benazepril impurity A IPRS ([(3R)-3-[[(1R)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2-oxo-2,3,4,5- tetrahydro-1H-1-benzazepin-1-yl]acetic acid IPRS)* in 50.0 ml of the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). A 0.01 per cent w/v solution of *benazepril hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.0 mm packed with silica gel a-1 acid glycoprotein for chiral chromatography (5 µm),
- mobile phase: a mixture of 20 volumes of *methanol* and 80 volumes of a buffer solution pH 6.0 prepared by dissolving 3.58 g of *disodium hydrogen phosphate* and

9.66 g of *potassium dihydrogen phosphate* in 1000 ml of *water*,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 50 µl.

The relative retention time with respect to benazepril (retention time: about 6.0 minutes) for benazepril impurity A is about 1.9.

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram for 3.5 times the retention time of the principal peak. The area of any peak corresponding to benazepril impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying under vacuum at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a) and test solution (b).

Calculate the content of $C_{24}H_{29}ClN_2O_5$.

Storage. Store protected from light and moisture.

Benazepril Hydrochloride Tablets

Benazepril Hydrochloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of benazepril hydrochloride, $C_{24}H_{28}N_2O_5$,HCl.

Usual strengths. 5 mg; 10 mg; 20 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 80 volumes of *ethyl acetate*, 20 volumes of *methanol* and 15 volumes of *ammonium hydroxide*.

Test solution. Disperse a quantity of powdered tablets containing 50 mg of Benazepril Hydrochloride in 30 ml of *methanol* with the aid of ultrasound for 15 minutes and dilute to 50.0 ml with *methanol*. Centrifuge and use the supernatant liquid.

Reference solution. A 0.1 per cent w/v solution of *benazepril hydrochloride IPRS* in *methanol*.

Apply to the plate 20 µl of each solution. After development dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve a weighted quantity of *benazepril hydrochloride IPRS* in *methanol* and dilute with dissolution medium to obtain a solution having a known concentration similar to the concentration of the test solution.

Use chromatographic system as described under Assay, using 60 µl injection volume:

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{28}N_2O_5$,HCl in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{24}H_{28}N_2O_5$,HCl.

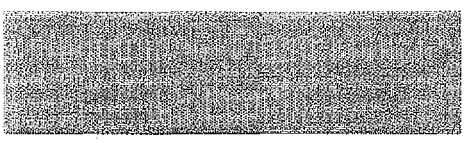
Related substances. Determine by liquid chromatography (2.4.14) as described under Assay with the following modification and using 80 µl injection volume:

Reference solution (a). A 0.0006 per cent w/v solution of *benazepril impurity C IPRS ((3-(1-carboxy-3-phenyl-1(S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H -1-(3S)-benzazepine-1-acetic acid IPRS)* in the mobile phase.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to benazepril impurity C is not more than 3.0 per cent. The area of any other secondary peak is not more than 1.0 per cent. The sum of the areas of all other secondary peaks is not more than 2.0 per cent, calculated by area normalization.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay using following test solution.



Test solution. Disperse 1 tablet in the mobile phase with the aid of ultrasound for 15 minutes and dilute to 50.0 ml with the mobile phase. Centrifuge and use the supernatant liquid.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Benazepril Hydrochloride in the 150 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 250.0 ml with the mobile phase. Centrifuge and use the supernatant liquid.

Reference solution (a). A 0.02 per cent w/v solution of *benazepril hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.04 per cent w/v each of *benazepril hydrochloride IPRS* and *benazepril impurity B IPRS* ((3*S*) 3-[[(1*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepene-1-acetic acid monohydrate) in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 64 volumes of *methanol* and 36 volumes of *tetrabutylammonium bromide solution*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 25 µl.

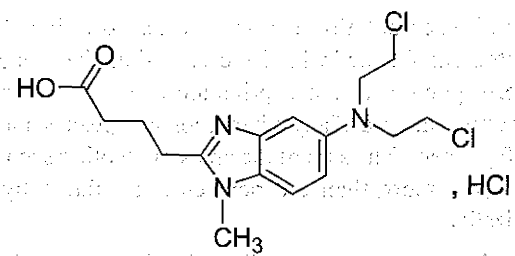
Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to benazepril hydrochloride and benazepril impurity B is not less than 1.7 and the relative standard deviation for replicate injection for each peak is not more than 2.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{24}H_{28}N_2O_5$,HCl in the tablets.

Storage. Store protected from moisture.

Bendamustine Hydrochloride



$C_{16}H_{21}Cl_2N_3O_2$, HCl, H_2O Mol Wt. 412.7

Bendamustine Hydrochloride is 4-[5-[bis(2-chloroethyl) amino]-1-methylbenzimidazol-2-yl]butanoic acid hydrochloride.

Bendamustine Hydrochloride contains not less than 98.5 per cent and not more than 102.0 per cent of $C_{16}H_{21}Cl_2N_3O_2$.HCl, calculated on the anhydrous basis.

Category. Antineoplastic.

Description. A white to almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bendamustine hydrochloride IPRS* or with the reference spectrum of bendamustine hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 0.5 per cent w/v solution (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).B

pH (2.4.24). 2.5 to 3.5, determined in solution A.

Chloride content. Between 9.0 per cent and 9.5 per cent, calculated on anhydrous basis, dissolve 0.4 g in 5 ml of *carbon dioxide-free water*, add 5 ml of *anhydrous glacial acetic acid* and 50 ml of *methanol*, and titrate with *0.1N silver nitrate* using *eosin solution* as indicator. Carry out a blank titration.

1 ml of *0.1N silver nitrate* is equivalent to 0.003545 g of Chloride.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20.0 mg of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol*.

Reference solution (a). A solution containing 0.02 per cent w/v of *bendamustine impurity A IPRS* in *methanol*.

Reference solution (b). A solution containing 0.02 per cent w/v of *bendamustine impurity B IPRS* in *methanol*.

Reference solution (c). A solution containing 0.02 per cent w/v of *bendamustine hydrochloride IPRS* in *methanol*.

Reference solution (d). Dilute 2.0 ml of reference solution (a), reference solution (b) and 1.0 ml of reference solution (c) to 100.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A, a 0.1 per cent v/v solution of *trifluroacetic acid* in *water*;
  B: *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
3	100	0
35	10	90
40	10	90
41	100	0
45	100	0

Name	Relative retention time
Impurity A[1]	0.75
Bendamustine	1.00
Impurity B[2]	1.13

[1]5-[Bis(2-hydroxyethyl)amino]-1-methyl-1H-bezimidazole-2-butanoic acid ethyl ester,

[2]5-[Bis (2-chloroethyl) amino]-1-methyl-1H-benzimidazole-2-butanoic acid ethyl ester.

Inject reference solution (d). The test is not valid unless the relative standard deviation for replicate injections of each peaks is not more than 5.0.

Inject reference solution (d) and test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to bendamustine Impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.2 per cent),the area of any peak corresponding to bendamustine Impurity B is not more than 2.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of any other secondary peak is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent) and the sum of area of all the impurities is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Bacterial endotoxins (2.2.3). Not more than 1.125 Endotoxin unit per mg of Bendamustine hydrochloride.

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 0.5 g.

Water (2.3.43). Not more than 0.5 per cent for the anhydrous form and 4.5 to 6.5 per cent for the monohydrate form determined on 0.1 gm.

Assay. Determine by liquid chromatography (2.4.14). as described under Related substances with the following modification.

– injection volume: 10 µl.

Test solution. Dissolve 20 mg of the substance under examination in methanol and dilute to 100.0 ml with *methanol.* Dilute 5.0 ml of the solution to 10.0 ml with *methanol.*

Reference solution. Dissolve 20 mg of *bendamustine hydrochloride IPRS* in *methanol* and dilute to 100.0 ml with *methanol.* Dilute 5.0 ml of the solution to 10.0 ml with *methanol.*

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{21}Cl_2N_3O_2,HCl$.

Storage. Store in airtight containers, protected from light at a temperature between 2° to 8°.

Bendamustine Injection

Bendamustine Hydrochloride Injection

Bendamustine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bendamustine hydrochloride $C_{16}H_{21}Cl_2N_3O_2.HCl$.

The constituted solution complies with the requirements for the Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

NOTE— Bendamustine hydrochloride is cytotoxic; extra care required to prevent inhaling particles and exposing the skin to it.

Usual strength. 100 mg.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powder for Injections) and with the following requirements.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 2.5 to 3.5, determined in a 0.5 per cent w/v solution in *carbon dioxide-free water.*

Related substances. Determine by liquid chromatography (2.4.14)

Test solution. Dissolve a quantity of powder to obtain a solution containing 0.1 per cent w/v of bendamustine hydrochloride in mobile phase B. Dilute 5.0 ml of the solution to 20.0 ml with *acetonitrile.*

Reference solution. A solution containing 0.025 per cent w/v of *bendamustine hydrochloride IPRS* in mobile phase B, dilute 5.0 ml of the solution to 50 ml with *acetonitrile*. Further dilute 5.0 ml of the solution to 50.0 ml with *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 90 volumes of a 0.1 per cent v/v solution of *trifluroacetic acid* in *water and* 10 volumes of *acetonitrile*,
  B. a mixture of 50 volumes of a 0.1 per cent v/v solution of *trifluroacetic acid* in *water and* 50 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl,

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
3	100	0
16	50	50
33	30	70
35	10	90
40	10	90
41	100	0
45	100	0

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Bacterial endotoxins (2.2.3). Not more than 2.25 Endotoxin Units per mg of bendamustine hydrochloride.

Water (2.3.43). Not more than 3.0 per cent.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances with the following modifications.

Reference solution. A solution containing 0.1 per cent w/v of *bendamustine hydrochloride IPRS* in mobile phase B. Dilute 5.0 ml of the solution to 20.0 ml with *acetonitrile*.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

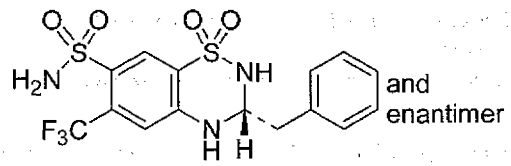
Calculate the content of $C_{16}H_{21}Cl_2N_3O_2.HCl$ in the injection.

Storage. Store protected from moisture at a temperature not exceeding 25°.

Labeling. The label states the strength in terms of the equivalent amount of Bendamustine hydrochloride.

Bendrofluazide

Bendroflumethiazide



$C_{15}H_{14}F_3N_3O_4S_2$ Mol. Wt. 421.4

Bendrofluazide is (3*RS*)-3-benzyl-6-(trifluoromethyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.

Bendrofluazide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{14}F_3N_3O_4S_2$, calculated on the dried basis.

Category. Diuretic.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bendrofluazide IPRS* or with the reference spectrum of bendrofluazide.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 2 g of *citric acid monohydrate* in 1000 ml of *water*.

Solvent mixture. 40 volumes of *methanol* and 60 volumes of buffer solution.

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dissolve 2 mg of *bendrofluazide impurity A IPRS (4-amino-6-(trifluoromethyl) benzene-1,3-*

disulfonamide) and 2.5 mg of *altizide IPRS* in the solvent mixture and dilute to 10.0 ml with the solvent mixture. Mix 1.0 ml of the solution with 1.0 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm × 3.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 15 volumes of *tetrahydrofuran*, 25 volumes of *methanol* and 60 volumes of buffer solution,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 273 nm,
- injection volume: 20 µl.

Name	Relative retention time
Bendrofluazide impurity A	0.2
Altizide	0.5
Bendrofluazide (Retention time is about 8 minutes)	1.0

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to bendrofluazide and altizide is not less than 10.0.

Inject reference solution (b) and the test solution. Run the chromatogram at least twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to bendrofluazide impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g, by drying in an oven at 105°.

Assay. Dissolve 0.15 g in 50.0 ml of *dimethyl sulphoxide*. Titrate with *0.1 M tetrabutylammonium hydroxide* in *2-propanol*, determining the end point potentiometrically (2.4.25). Read the volumes added between the two points of inflection.

1.0 ml of *0.1 M tetrabutylammonium hydroxide* in *2-propanol* is equivalent to 0.02107 g of $C_{15}H_{14}F_3N_3O_4S_2$.

Bendrofluazide Tablets

Bendroflumethiazide Tablets

Bendrofluazide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of bendrofluazide, $C_{15}H_{14}F_3N_3O_4S_2$.

Usual strength. 2.5 mg.

Identification

A. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Ethyl acetate.

Test solution. Disperse a quantity of the powdered tablets containing 10 mg of bendrofluazide with 10.0 ml of *acetone*, with the aid of ultrasound for 10 minutes and filter.

Reference solution. A 0.1 per cent w/v solution of *bendrofluazide IPRS* in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Ethyl acetate.

Test solution. Disperse a quantity of the powdered tablets containing 25 mg of bendrofluazide with 25 ml of *acetone*, with the aid of ultrasound for 10 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 2.5 ml of *acetone*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 15 mg of Bendrofluazide with 50 ml of *0.1M sodium hydroxide* with the aid of ultrasound for 10 minutes and add sufficient *0.1M sodium hydroxide* to produce 100.0 ml, filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7).

Calculate the content of $C_{15}H_{14}F_3N_3O_4S_2$ taking 410 as the specific absorbance at 275 nm.

Bentonite

Bentonite is a natural, colloidal, hydrated aluminium silicate that has been processed to remove grit and non-swelling components of the ore.

Category. Pharmaceutical aid (suspending agent).

Description. A very fine, pale buff or cream-coloured to greyish-white powder, free or almost free from gritty particles.

Identification

Fuse 1 g with 2 g of *anhydrous sodium carbonate*, warm the residue with 10 ml of *water*, filter, wash the filter with 5 ml of *water* and reserve the combined filtrate and washings. Dissolve the residue in 10 ml of *dilute hydrochloric acid*; the solution gives the reactions of aluminium salts, (2.3.1). Add to the reserved filtrate and washings 3 ml of *hydrochloric acid*; a gelatinous precipitate is produced.

Tests

pH (2.4.24). 9.0 to 10.5, determined in a 2.0 per cent w/v suspension in *water*.

Sedimentation volume. In a mortar, mix 6.0 g with 0.3 g of *light magnesium oxide*, freshly calcined. Mix the powder progressively with 200 ml of *water*. Shake for 1 hour and place 100 ml of the suspension in a 100-ml graduated cylinder. After 24 hours the volume of the clear supernatant liquid is not greater than 2 ml.

Swelling power. Add 2.0 g in twenty portions at intervals of 2 minutes to 100 ml of a 1 per cent w/v solution of *sodium lauryl sulphate* in a 100-ml graduated cylinder about 3 cm in diameter. Allow each portion to settle before adding the next and let it stand for 2 hours. The apparent volume of the sediment at the bottom of the cylinder is not less than 24 ml.

Coarse particles. To 20 g add 1000 ml of *water* and mix for 15 minutes at not less than 5000 rpm. Transfer to a wet sieve of nominal aperture of 75 mm, previously dried at 100° to 105° and weighed, and wash with three quantities, each of 500 ml, of *water*, ensuring that any agglomerates are dispersed. Dry at 100° to 105° and weigh. The weight of the matter on the sieve is not more than 0.1 g (0.5 per cent).

Heavy metals (2.3.13). To 5.0 g add 7.5 ml of *2 M hydrochloric acid* and 27.5 ml of *water*, boil for 5 minutes, centrifuge and filter the supernatant liquid. Wash the residue with *water*, filter, combine the filtrates and dilute to 50 ml with *water*. To 5 ml of the solution add 5 ml of *water*, 10 ml of *hydrochloric acid* and 25 ml of *4-methyl-2-pentanone*, shake for 2 minutes, allow the layers to separate and evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *5 M acetic acid*, dilute to 25 ml and filter. The resulting solution complies with the limit test for heavy metals, Method D (50 ppm). Prepare the standard using *lead standard solution (1 ppm Pb)*.

Microbial contamination (2.2.9). 1g is free from *Escherichia coli*.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Benzalkonium Chloride

Benzalkonium Chloride is a mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups having chain lengths of C_8 to C_{18}.

Benzalkonium Chloride contains not less than 95.0 per cent and not more than 104.0 per cent of alkylbenzyldimethylammonium chlorides, calculated as $C_{22}H_{40}ClN$ on the anhydrous basis.

Category. Antiseptic.

Description. A white or yellowish-white powder or gelatinous, yellowish-white fragments, hygroscopic, soapy to the touch.

Identification

A. Dilute 0.1 g with 10 ml of *water*. To 5 ml add 1.5 ml of *dilute nitric acid*; a white precipitate is produced which is soluble in *ethanol (95 per cent)*. To the remainder add 1.5 ml of *mercuric chloride solution*; a white precipitate is produced which is soluble in *ethanol (95 per cent)*.

B. Dissolve 0.25 g in 1 ml of *sulphuric acid*, add 0.1 g of *potassium nitrate*, heat on a water-bath for 5 minutes, cool, dilute with *water* to 10 ml, add 0.5 g of *zinc powder*, and heat on a water-bath for 5 minutes. To 2 ml of the clear supernatant liquid add 0.5 ml of *sodium nitrite solution*, cool in ice and add to 3 ml of *2-naphthol solution*; an orange red colour is produced.

C. To 25 mg add 1 ml of *2 M nitric acid*; a white precipitate is produced which dissolves on addition of 5 ml of *ethanol (95 per cent)*. The resulting solution gives reaction (A) of chlorides (2.3.1).

Tests

Acidity or alkalinity. Dissolve 0.5 g in 50 ml of *carbon dioxide-free water*, add 0.1 ml of *bromocresol purple solution* and titrate with *0.1 M hydrochloric acid* or with *0.1 M sodium hydroxide*. Not more than 0.1 ml is required to change the colour of the solution.

Ammonia compounds. Boil 0.1 g with 3 ml of *sodium hydroxide solution*; no odour of ammonia is produced.

Foreign amines. Dissolve 0.1 g in 5 ml of *water* and add 3 ml of *1 M sodium hydroxide*; no precipitate is formed. Heat to boiling; the odour of amines is not perceptible.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 10 per cent, determined on 0.3 g.

Assay. Weigh 2.0 g, dissolve in sufficient *water* to produce 100.0 ml. Transfer 25.0 ml to a separating funnel, add 25 ml of *chloroform*, 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 5 per cent w/v solution of *potassium iodide*. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous solution with three further quantities, each of 10 ml, of *chloroform* and discard the chloroform layer. Add 40 ml of *hydrochloric acid*, cool and titrate with *0.05 M potassium iodate* until the solution becomes pale brown in colour. Add 2 ml of *chloroform* and continue the titration until the chloroform becomes colourless. Titrate a mixture of 20 ml of *water*, 10.0 ml of a freshly prepared 5 per cent w/v solution of potassium iodide and 40 ml of *hydrochloric acid* with *0.05 M potassium iodate* in a similar manner; the difference between the titrations represents the amount of *0.05 M potassium iodate* required.

1 ml of *0.05 M potassium iodate* is equivalent to 0.0354 g of $C_{22}H_{40}ClN$.

Storage. Avoid contact with metals.

Benzalkonium Chloride Solution

Benzalkonium Chloride Solution is a solution of a mixture of alkylbenzyldimethylammonium chlorides, the alkyl groups having chain lengths of C_8 to C_{18}. It may contain *ethanol (95 per cent)*. In making Benzalkonium Chloride Solution, the *ethanol (95 per cent)* may be replaced by Industrial Methylated Spirit, diluted so as to be of equivalent strength.

Benzalkonium Chloride Solution contains not less than 49.0 per cent w/v and not more than 51.0 per cent w/v of alkylbenzyldimethylammonium chlorides, calculated as $C_{22}H_{40}ClN$. It may contain not more than 16.0 per cent v/v of ethanol, C_2H_6O.

Category. Antiseptic detergent.

Description. A clear, colourless or slightly yellow, syrupy liquid.

Identification

A. Dilute 0.2 ml with 10 ml of *water*. To 5 ml add 1.5 ml of *dilute nitric acid*; a white precipitate is produced which is soluble in *ethanol (95 per cent)*. To the remainder add 1.5 ml of *mercuric chloride solution*; a white precipitate is produced which is soluble in *ethanol (95 per cent)*.

B. Evaporate 0.5 ml to dryness on a water-bath, dissolve the residue in 1 ml of *sulphuric acid*, add 0.1 g of *potassium nitrate*, heat on a water-bath for 5 minutes, cool, dilute with *water* to 10 ml, add 0.5 g of *zinc powder*, and heat on a water-bath for 5 minutes. To 2 ml of the clear supernatant liquid add 0.5 ml of *sodium nitrite solution*, cool in ice and add to 3 ml of *2-naphthol solution*; an orange red colour is produced.

C. To 0.05 ml add 1 ml of *2 M nitric acid*; a white precipitate is produced which dissolves on addition of 5 ml of *ethanol (95 per cent)*. The resulting solution gives reaction (A) of chlorides (2.3.1).

Tests

Acidity or alkalinity. Dissolve 1.0 g in 50 ml of *carbon dioxide-free water*, add 0.1 ml of *bromocresol purple solution* and titrate with *0.1 M hydrochloric acid* or with *0.1 M sodium hydroxide*. Not more than 0.1 ml is required to change the colour of the solution.

Ammonia compounds. Boil 0.2 ml with 3 ml of *sodium hydroxide solution*; no odour of ammonia is produced.

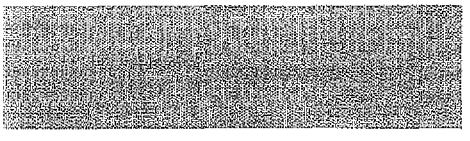
Foreign amines. To a volume containing 0.1 g of benzalkonium chloride add sufficient *water* to produce 5 ml and add 3 ml of *1 M sodium hydroxide*; no precipitate is formed. Heat to boiling; the odour of amines is not perceptible.

Ethanol *(if present)* (2.3.45). Not more than 16.0 per cent v/v, determined by Method I or II, as applicable.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Assay. Weigh 4.0 g, dissolve in sufficient *water* to produce 100.0 ml. Transfer 25.0 ml to a separating funnel, add 25 ml of *chloroform*, 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 5 per cent w/v solution of *potassium iodide*. Shake well, allow to separate and discard the chloroform layer. Shake the aqueous solution with three further quantities, each of 10 ml, of *chloroform* and discard the chloroform layer. Add 40 ml of *hydrochloric acid*, cool and titrate with *0.05 M potassium iodate* until the solution becomes pale brown in colour. Add 2 ml of *chloroform* and continue the titration until the chloroform becomes colourless. Titrate a mixture of 20 ml of *water*, 10.0 ml of a freshly prepared 5.0 per cent w/v solution of *potassium iodide* and 40 ml of *hydrochloric acid* with *0.05 M potassium iodate* in a similar manner; the difference between the titrations represents the amount of *0.05 M potassium iodate* required.

1 ml of *0.05 M potassium iodate* is equivalent to 0.0354 g of $C_{22}H_{40}ClN$. Determine the relative density (2.4.29), and calculate the amount of $C_{22}H_{40}ClN$, weight in volume.

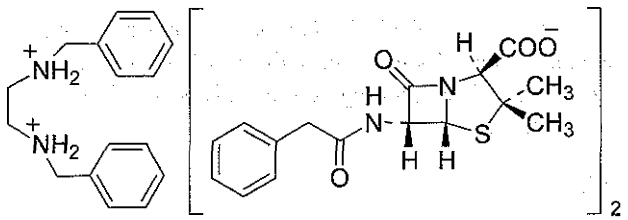


Storage. Avoid contact with metals.

Labelling. The label states, where appropriate, the content of *ethanol (95 per cent)* or Industrial Methylated Spirit.

Benzathine Penicillin

Benzathine Benzylpenicillin; Benzathine Penicillin G



$C_{16}H_{20}N_2,(C_{16}H_{18}N_2O_4S)_2$ Mol. Wt. 909.0

Benzathine Penicillin is *N,N'*-dibenzylethylenediammonium bis[(6*R*)-6-(2-phenylacetamido)penicillanate] containing a variable amount of water.

Benzathine Penicillin contains not less than 96.0 per cent and not more than 100.5 per cent of $C_{16}H_{20}N_2,(C_{16}H_{18}N_2O_4S)_2$ and not less than 24.0 per cent and not more than 27.0 per cent of $C_{16}H_{20}N_2$, both calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white, crystalline powder; almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzathine penicillin IPRS*.

B. Shake 0.1 g with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *potassium dichromate solution*; a golden yellow precipitate is formed.

C. Shake 0.1 g with 2 ml of *1 M sodium hydroxide* for 2 minutes, extract the mixture with two quantities, each of 3 ml, of *ether*, evaporate the combined extracts and dissolve the residue in 1 ml of *ethanol (50 per cent)*. Add 5 ml of *picric acid solution*, heat at 90° for 5 minutes and allow to cool slowly; the precipitate, after recrystallisation from *ethanol (25 per cent)* containing a small quantity of *picric acid*, melts at about 214° (2.4.21).

D. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 5.0 to 7.5, determined in a saturated solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use. Avoid any overheating during the preparation of the solutions.

Test solution. Dissolve a weighed quantity of about 70 mg of the substance under examination in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.

Reference solution (a). Dissolve a weighed quantity of about 70 mg of *benzathine penicillin IPRS* in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *orthophosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,
  B. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *orthophosphoric acid*, 30 volumes of *water* and 60 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
10	75	25
20	0	100
55	0	100
70	75	25

Inject reference solution (a). The relative retention time with reference to benzylpenicillin for benzathine is about 0.3 to 0.4;

for benzylpenicilloic acids benzathide is about 2.4. If necessary, adjust the concentration of *methanol* in the mobile phase.

Inject reference solution (b) and the test solution. The area of any secondary peak obtained with the test solution corresponding to benzylpenicilloic acid benzathide is not more than twice the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (2 per cent).The area any other secondary peak obtained with the test solution is not more than the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (1 per cent). Disregard any peak with an area 0.05 times the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (2.3.43). 5.0 to 8.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography, (2.4.14) as given under the test for Related substances using the following mobile phase.

Mobile phase. a mixture of 10 volumes of *phosphate buffer solution pH 3.5,* 35 volumes of *methanol,* and 55 volumes of *water.*

Inject reference solution (a) and the test solution.

Calculate the contents of $C_{16}H_{20}N_2$ and of $C_{16}H_{20}N_2$, $(C_{16}H_{18}N_2O_4S)_2$. Calculate the content of $C_{16}H_{20}N_2$, $(C_{16}H_{18}N_2O_4S)_2$ by multiplying the percentage content of benzylpenicillin by 1.36.

Benzathine Penicillin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.13 Endotoxin Unit per ml of a solution prepared by suspending 20 mg of the substance under examination in 20 ml of *0.1 M sodium hydroxide* diluted 1 ml to 100 ml and using the supernatant.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If the material is intended for use in the manufacture of parenteral preparations the container should be sterile and sealed so as to exclude micro-organisms.

Labelling The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Benzathine Penicillin Injection

Benzathine Benzylpenicillin Injection; Benzathine Penicillin G Injection

Benzathine Penicillin Injection is a sterile material consisting of Benzathine Penicillin with or without suspending agents, buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by suspending the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

Usual strength. 450 mg (600,000 Units) (Each mg of Benzathine Penicillin is approximately equivalent to 1,330 Units of penicillin).

Storage. The constituted suspension should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Benzathine Penicillin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of benzathine penicillin, $C_{16}H_{20}N_2$, $(C_{16}H_{18}N_2O_4S)_2$.

Description. A white crystalline powder, almost odourless.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Shake 0.1 g with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *potassium dichromate solution*; a golden yellow precipitate is formed.

B. Shake 0.1 g with 2 ml of *1 M sodium hydroxide* for 2 minutes, extract the mixture with two quantities, each of 3 ml, of *ether,* evaporate the combined extracts and dissolve the residue in 1 ml of *ethanol (50 per cent)*. Add 5 ml of *picric acid solution*, heat at 90° for 5 minutes and allow to cool slowly; the precipitate, after recrystallisation from *ethanol (25 per cent)* containing a small quantity of *picric acid*, melts at about 214° (2.4.21).

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 5.0 to 7.5, determined in a suspension obtained by reconstituting as directed on the label.

Consistency. To a quantity containing 60,000 Units add 2 ml of *water* and shake thoroughly. The resulting suspension passes through a 23G hypodermic needle.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use. Avoid any overheating during the preparation of the solutions.

Test solution. Dissolve a weighed quantity containing about 70 mg of Benzathine Penicillin in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.

Reference solution (a). Dissolve a weighed quantity of about 70 mg of *benzathine penicillin IPRS* in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *orthophosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,
  B. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *orthophosphoric acid*, 30 volumes of *water* and 60 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
10	75	25
20	0	100
55	0	100
70	75	25

Inject reference solution (a). The relative retention time with reference to benzylpenicillin for benzathine is about 0.3 to 0.4; for benzylpenicilloic acids benzathide is about 2.4.

Inject reference solution (b) and the test solution. The area of any secondary peak obtained with the test solution corresponding to benzylpenicilloic acids benzathide is not more than twice the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (2.0 per cent). The area of any other secondary peak obtained with the test solution is not more than the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (1 per cent). Disregard any peak with an area 0.05 times the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

Bacterial endotoxin (2.2.3). Not more than 0.13 Endotoxin Unit per ml of a solution prepared by suspending 20 mg of the substance under examination in 20 ml of *0.1 M sodium hydroxide* diluted 1 ml to 100 ml and using the supernatant.

Water (2.3.43). 5.0 to 8.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography, (2.4.14) as given under the test for Related substances using the following mobile phase.

Mobile phase. a mixture of 10 volumes of *phosphate buffer solution pH 3.5*, 35 volumes of *methanol*, and 55 volumes of *water*.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{20}N_2S(C_{16}H_{18}N_2O_4S)_2$ by multiplying the percentage content of benzylpenicillin by 1.36.

Labelling. The label states (1) the directions for constituting the suspension; (2) the names of any added buffering agents or other pharmaceutical aids; (3) that the preparation is meant for intramuscular injection only.

Fortified Benzathine Penicillin Injection

Fortified Benzathine Benzylpenicillin Injection; Fortified Benzathine Penicillin G Injection

Benzathine Penicillin Injection is a sterile material consisting of Benzathine Penicillin and Procaine Penicillin with or without suspending agents, buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by suspending the contents of the sealed container in the requisite amount of sterile Water for Injections containing Benzylpenicillin Sodium immediately before use.

Storage. The constituted suspension should be used immediately after preparation but, in any case, within the period and under the conditions recommended by the manufacturer.

Fortified Benzathine Penicillin Injection contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of benzathine penicillin, not less than 95.0 per cent and not more than 125.0 per cent of the stated amount of procaine penicillin, not less than 90.0 per cent and not more than 130.0 per cent of the stated amount of benzylpenicillin sodium, all in terms of Units of penicillin.

Category. Antibacterial.

Usual strength. Benzathine Penicillin, 450 mg (600,000 Units), Procaine Penicillin, 300 mg (300,000 Units) and Benzylpenicillin, 180 mg (300,000 Units).

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. It gives the reaction for penicillins (2.3.1).

B. It gives reaction (B) of penicillins and cephalosporins (2.3.1).

C. Shake 0.1 g with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *potassium dichromate solution*; a golden yellow precipitate is formed.

D. It give the reactions of sodium salts (2.3.1).

Tests

Stability. Using an aseptic technique prepare the suspension as directed on the label in an individual unopened container and determine the concentration of benzylpenicillin sodium by the method described below using a measured quantity of the suspension, withdrawn aseptically from the container. Store the remainder of the suspension in the closed container at 4° for 7 days and then repeat the determination of benzylpenicillin sodium.

The concentration of benzylpenicillin sodium in the stored injection is not less than 80 per cent of the concentration found in the freshly prepared suspension.

Consistency. To a quantity containing 600,000 Units of Benzathine Penicillin, 300,000 Units each of Procaine Penicillin and Benzylpenicillin add 2 ml of *water* and shake thoroughly. The resulting suspension passes readily through a 22G hypodermic needle.

Bacterial endotoxin (2.2.3). Not more than 0.13 Endotoxin Unit per ml of a solution prepared by suspending 20 mg of the substance under examination in 20 ml of *0.1 M sodium hydroxide* diluted 1 ml to 100 ml and using the supernatant.

Water (2.3.43). Not more than 7.5 per cent, determined on 0.3 g.

Assay. *For benzathine penicillin* — Disperse a quantity of the mixed contents of 10 containers containing 1 g of Benzathine Penicillin with 30 ml of a saturated solution of *sodium chloride* and 10 ml of *5 M sodium hydroxide* and extract with four successive quantities, each of 50 ml of *ether*. Wash the combined ether extracts with three successive quantities, each of 5 ml, of *water*, extracting each aqueous washing with the same 25 ml of *ether*. Combine the ether extracts, evaporate to a low bulk, add 2 ml of *ethanol* and evaporate to dryness. Dissolve the residue in 50 ml of *glacial acetic acid* and titrate with *0.1 M perchloric acid* using 1 ml of *1-naphtholbenzein solution* as indicator.

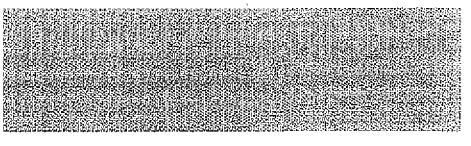
1 ml of *0.1 M perchloric acid* is equivalent to 0.04545 g of $C_{18}H_{50}N_6O_8S_2$. Calculate the apparent content of Benzathine Penicillin.

Calculate the content of procaine penicillin, as determined by the method given below in the weight of the sample used in this assay, multiply this content by a factor of 1.544 and deduct the figure from the apparent content of benzathine penicillin; the result is the content of benzathine penicillin. (1 mg of benzathine penicillin is approximately equivalent to 1330 Units of penicillin).

For procaine penicillin — To a quantity of the mixed contents of 10 containers containing 0.25 g of Procaine Penicillin add 100 ml of *water*, shake well, dilute to 200.0 ml with *water*, mix and filter. Dilute 5.0 ml of the filtrate to 250.0 ml with *buffer solution pH 7.0* and measure the absorbance of the resulting solution at the maximum at about 290 nm, using *buffer solution pH 7.0* as the blank (2.4.7). Calculate the content of procaine penicillin taking 310 as the specific absorbance at 290 nm. (1 mg of procaine penicillin is equivalent to 1009 Units of penicillin).

For benzylpenicillin sodium — Disperse a quantity of the mixed contents of 10 containers containing 0.15 g of Benzylpenicillin Sodium with water until dissolved and dilute to 500.0 ml with *water*. Dilute 25.0 ml of the resulting solution to 100.0 ml with *phosphate buffer pH 6.8*. Place two quantities, each of 2.0 ml, of the resulting solution in separate stoppered tubes. To one tube add 10.0 ml of *imidazole-mercury reagent*, mix, stopper the tube and immerse in a water-bath at 60° for 35 minutes, swirling occasionally. Remove from the water-bath and cool rapidly to 20° (solution A). Add 10.0 ml of *imidazole solution* to the second tube, mix, stopper the tube and allow to stand at 20° for 35 minutes, swirling occasionally (solution B). Without delay measure the absorbance of solutions A and B at about 325 nm (2.4.7), using as the blank a mixture of 2.0 ml of *water* and 10.0 ml of *imidazole-mercury reagent* for solution A and a mixture of 2.0 ml of *water* and 10.0 ml of *imidazole solution* for solution B. Calculate the content of total penicillins as $C_{16}H_{17}N_2NaO_4S$ from the difference between the absorbances of solutions A and B, from the difference obtained by repeating the procedure using 0.15 g of *benzylpenicillin sodium IPRS* in place of the contents of the sealed containers. Calculate the content of benzylpenicillin sodium by subtracting the contents of benzathine penicillin and procaine penicillin, both expressed as benzylpenicillin sodium, $C_{16}H_{17}N_2NaO_4S$.

Labelling. The label on the sealed container states (1) the quantity of Benzathine Penicillin, Benzylpenicillin Sodium and Procaine Penicillin contained in it; (2) the directions for



reconstituting the suspension; (3) the names of the added suspending agent, buffering agent and any other pharmaceutical aid; (4) that the preparation is intended for intramuscular injection only.

Benzathine Penicillin Tablets

Benzathine Benzylpenicillin Tablets; Benzathine Penicillin G Tablets

Benzathine Penicillin Tablets contain Benzathine Penicillin equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of penicillin.

Usual strength. 200,000 Units of penicillin.

Identification

A. Shake 0.1 g with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *potassium dichromate solution*; a golden yellow precipitate is formed.

B. Shake 0.1 g with 2 ml of *1 M sodium hydroxide* for 2 minutes, extract the mixture with two quantities, each of 3 ml, of *ether*, evaporate the combined extracts and dissolve the residue in 1 ml of *ethanol (50 per cent)*. Add 5 ml of *picric acid solution*, heat at 90° for 5 minutes and allow to cool slowly; the precipitate, after recrystallisation from *ethanol (25 per cent)* containing a small quantity of *picric acid*, melts at about 214° (2.4.21).

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use. Avoid any overheating during the preparation of the solutions.

Test solution. Weigh and powder 20 tablets. Dissolve a weighed quantity containing about 70 mg of Benzathine Penicillin in 25 ml of *methanol* with the aid of ultrasound for 2 minutes and allow to stand for 15 minutes. Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate* mix and filter.

Reference solution (a). Dissolve a weighed quantity of about 70 mg of *benzathine penicillin IPRS* in 25 ml of *methanol* with the aid of ultrasound (for about 2 minutes). Dilute to 50.0 ml with a solution containing 6.8 g per litre of *potassium dihydrogen phosphate* and 1.02 g per litre of *disodium hydrogen phosphate*.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *orthophosphoric acid*, 30 volumes of methanol and 60 volumes of *water*,
  B. a mixture of 10 volumes of a 34 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with *orthophosphoric acid*, 30 volumes of *water* and 60 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
10	75	25
20	0	100
55	0	100
70	75	25

Inject reference solution (a). The relative retention time with reference to benzylpenicillin for benzathine is about 0.3 to 0.4; for benzylpenicilloic acids benzathide is about 2.4. If necessary, adjust the concentration of *methanol* in the mobile phase.

Inject reference solution (b) and the test solution. The area of any secondary peak obtained with the test solution corresponding to benzylpenicilloic acids benzathide is not more than twice the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (2.0 per cent). The area of any other secondary peak obtained with the test solution is not more than the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area 0.05 times the sum of the areas of the two principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (2.3.43). Not more than 8.0 per cent, determined on the powdered tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography, (2.4.14) as given under the test for Related substances using the following mobile phase.

Mobile phase. a mixture of 10 volumes of *phosphate buffer pH 3.5*, 35 volumes of *methanol*, and 55 volumes of *water*.

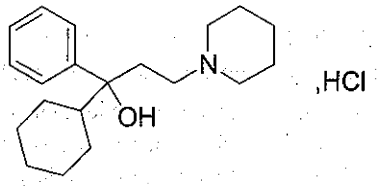
Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{20}N_2,(C_{16}H_{18}N_2O_4S)_2$ by multiplying the content of benzylpenicillin by 1.36.

Storage. Store at a temperature not exceeding 30°.

Benzhexol Hydrochloride

Trihexyphenidyl Hydrochloride



$C_{20}H_{31}NO,HCl$ Mol. Wt. 337.9

Benzhexol Hydrochloride is (*RS*)-1-cyclohexyl-1-phenyl-3-piperidinopropan-1-ol hydrochloride.

Benzhexol Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{20}H_{31}NO,HCl$, calculated on the dried basis.

Category. Antiparkinsonian.

Description. A white or creamy-white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzhexol hydrochloride IPRS* or with the reference spectrum of benzhexol hydrochloride.

B. Dissolve 0.5 g in 5 ml of warm *methanol* and make just alkaline to *litmus paper* with *5 M sodium hydroxide*; a precipitate is produced, which, after recrystallisation from *methanol* melts at about 114° (2.4.21).

C. It gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 5.2 to 6.2, determined in a solution prepared by dissolving 1.0 g in 50 ml of *carbon dioxide-free water* with the aid of heat, cooling and diluting to 100.0 ml with the same solvent.

Optical rotation (2.4.22). – 0.10° to + 0.10°, determine in a 5.0 per cent w/v solution in a mixture of 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 10.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 10 mg of *1-phenyl-3-(piperidin-1-yl)propan-1-one IPRS (trihexyphenidyl impurity A IPRS)* in 10.0 ml of the mobile phase.

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d). Add 1.0 ml of the test solution to 1.0 ml of the reference solution (b) and dilute to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 200 volumes of *water*, 0.2 volume of *triethylamine*, adjusted to pH 4.0 with *orthophosphoric acid* and 800 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to trihexyphenidyl and trihexyphenidyl impurity A not less than 3.0.

Inject reference solution (a), (c) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of secondary peak corresponding to trihexyphenidyl impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.1 per cent) and the sum of the areas of all secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.7 g in 50 ml of *anhydrous glacial acetic acid* previously neutralised using *1-naphtholbenzein solution* as indicator, warming and cooling, if necessary. Add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*

to the full colour change of the indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03379 g of $C_{20}H_{31}NO,HCl$.

Benzhexol Tablets

Benzhexol HydrochlorideTablets; Trihexyphenidyl Hydrochloride Tablets

Benzhexol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of benzhexol hydrochloride, $C_{20}H_{31}NO,HCl$.

Usual strengths. 2 mg; 5 mg.

Identification

A. Disperse a quantity of the powdered tablets containing 20 mg of benzhexol hydrochloride with 20 ml of *water* and filter. The filtrate yields a yellow precipitate with *trinitrophenol solution* and a white precipitate with *5 M sodium hydroxide*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Disperse a quantity of the powdered tablets with sufficient *chloroform* to produce a solution containing 0.2 per cent w/v of Benzhexol Hydrochloride and filter.

Reference solution. A 0.2 per cent w/v solution of *benzhexol hydrochloride IPRS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development remove the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Disperse one tablet in few ml of *water* with the aid of ultrasound and dilute with the mobile phase to obtain a solution containing 0.008 per cent w/v of benzhexol hydrochloride.

Reference solution. A solution containing 0.008 per cent w/v of *benzhexol hydrochloride IPRS* and 0.004 per cent w/v of *3-piperidylpropiophenone hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: 800 volumes of *acetonitrile*, 200 volumes of *water* and 0.2 volume of *triethylamine*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is not less than 4.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{31}NO,HCl$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 5 mg of Benzhexol Hydrochloride with a few ml of *water*, add 10 ml of the mobile phase, shake for 15 minutes, dilute to 25.0 ml with the mobile phase, mix and filter.

Reference solution. A solution containing 0.02 per cent w/v of *benzhexol hydrochloride IPRS* and 0.01 per cent w/v of *3-piperidylpropiophenone hydrochloride IPRS* in the mobile phase.

Chromatographic system

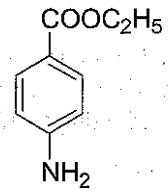
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 450 volumes of *acetonitrile*, 550 volumes of *water* and 5.0 volume of *triethylamine*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is not less than 4.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{31}NO,HCl$ in the tablets.

Benzocaine



$C_9H_{11}NO_2$ Mol. Wt. 165.2

Benzocaine is ethyl 4-aminobenzoate.

Benzocaine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_9H_{11}NO_2$, calculated on the dried basis.

Category. Local anaesthetic.

Description. Colourless crystals or a white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzocaine IPRS* or with the reference spectrum of benzocaine.

B. Dissolve 10 mg in 1 ml of *water* with the aid of one drop of *dilute hydrochloric acid* and add 2 drops of a 10 per cent w/v solution of *sodium nitrite* and 2 drops of a solution of 10 mg of *2-naphthol* in 5 ml of *sodium hydroxide solution*; a deep red colour is produced. On setting aside the solution for some time, a scarlet precipitate is produced.

C. Dissolve 0.2 g in 10 ml of *water* with the aid of *dilute hydrochloric acid* (solution A) and divide into 2 parts. To one part of solution A add *iodine solution*; a precipitate is obtained (distinction from orthocaine).

D. To the other part of solution A add *potassium mercuri-iodide solution*; no precipitate is obtained (distinction from procaine).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. Dissolve 0.5 g in 5 ml of *ethanol (95 per cent)*, add 10 ml of *water* and one drop of *phenolphthalein solution*; no pink colour is produced. Add 0.5 ml of *0.01 M sodium hydroxide*; the solution develops a pink colour.

Chlorides. Dissolve 0.2 g in 5 ml of *ethanol (95 per cent)* previously acidified with a few drops of *dilute nitric acid* and add few drops of *silver nitrate solution*; no turbidity is produced immediately.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

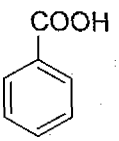
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa.

Assay. Weigh 0.4 g and dissolve in a mixture of 25 ml of *hydrochloric acid* and 50 ml of *water*, add 3g of *potassium bromide*. Cool to 10°. Determine by the nitrite titration (2.3.31). 1 ml of *0.1 M sodium nitrite* is equivalent to 0.01652 g of $C_9H_{11}NO_2$.

Storage. Store protected from light.

Benzoic Acid



$C_7H_6O_2$ Mol. Wt. 122.1

Benzoic Acid contains not less than 99.5 per cent and not more than 100.5 per cent of $C_7H_6O_2$, calculated on the anhydrous basis.

Category. Antifungal agent; pharmaceutical aid (anti- microbial preservative).

Description. Colourless, light crystals, scales or needles.

Identification

A. Melting point (2.4.21). 121° to 124°.

B. A 5.0 per cent w/v solution in *ethanol (95 per cent)*, gives reaction (a) of benzoates (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

Arsenic (2.3.10). Mix 5.0 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Readily oxidisable substances. Add 1 ml of *sulphuric acid* to 100 ml of *water*, heat to boiling and add dropwise *0.1 M potassium permanganate* until the pink colour persists for 30 seconds. Dissolve exactly 1 g in the hot solution and titrate with *0.1 M potassium permanganate* to a pink colour that persists for 15 seconds; not more than 0.5 ml of *0.1 M potassium permanganate* is required.

Readily carbonisable substances. Dissolve 0.5 g in 5 ml of *sulphuric acid* and allow to stand for 5 minutes. The colour of the solution is not more intense than that of reference solution YS5 (2.4.1).

Cinnamic acid. Warm 0.1 g with 0.1 g of *potassium permanganate* and 5 ml of *dilute sulphuric acid*; no odour of benzaldehyde is developed.

Chlorinated compounds. Dissolve 0.33 g in 5 ml of *0.5 M sodium carbonate*, evaporate to dryness and heat the residue until completely charred, keeping the temperature below 400°. Extract the residue with a mixture of 10 ml of *water* and 12 ml of *dilute nitric acid* and filter; the filtrate complies with the limit test for chlorides (2.3.12).

Heavy metals. Not more than 10 ppm, determined by the following method. Dissolve 2.0 g in 25 ml of *acetone* and add 2 ml of *water* and 10 ml of *hydrogen sulphide solution*; any colour produced is not more intense than that of a solution prepared with 25 ml of *acetone*, 2.0 ml of *lead standard solution (10 ppm Pb)* and 10 ml of *hydrogen sulphide solution*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.7 per cent, determined on 0.25 g and using a mixture of 1 volume of *methanol* and 2 volumes of *pyridine* as the solvent.

Assay. Dissolve 1.0 g in 15 ml of warm *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution*. Add 20 ml of *water* and titrate with *0.5 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.06106 g of $C_7H_6O_2$.

Compound Benzoic Acid Ointment

Benzoic and Salicylic Acids Ointment; Whitfield's Ointment

Compound Benzoic Acid Ointment is an ointment containing 6.0 per cent w/w of Benzoic Acid and 3.0 per cent w/w of Salicylic Acid in a suitable ointment base. Other strengths may also be prepared with Benzoic Acid and Salicylic Acid being in the ratio of about 2 to 1.

Compound Benzoic Acid Ointment contains not less than 5.7 per cent and not more than 6.3 per cent w/w of benzoic acid, $C_7H_6O_2$, and not less than 2.85 per cent and not more than 3.15 per cent w/w of salicylic acid, $C_7H_6O_3$.

Category. Antifungal (topical).

Identification

Carry out the method for thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid*.

Test solution. Warm 1 g of the ointment with 10 ml of *chloroform*, cool and filter.

Reference solution. A solution containing 0.6 per cent w/v of *benzoic acid* and 0.3 per cent w/v of *salicylic acid* in *chloroform*.

Apply to the plate 2 µl of each solution. After development, dry the plate in a current of air and examine under ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution. Examine the plate under ultraviolet light at 365 nm. A blue fluorescent spot in the chromatogram obtained with the test solution corresponds in colour and position to the one in the chromatogram obtained with the reference solution. Spray the plate with *ferric chloride test-solution*. The chromatogram obtained with the test solution shows a purple spot corresponding in position to the blue fluorescent spot observed in ultraviolet light at 365 nm and corresponding in colour and position to the spot in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Ointments.

Assay. *For benzoic acid* — Dissolve 2 g, in 150 ml of *water*, with the aid of gentle heat and titrate with *0.1M sodium hydroxide* using *phenolphthalein solution* as indicator. Reserve the solution for the Assay for salicylic acid.

1 ml of *0.1 M sodium hydroxide*, after deducting 1 ml for each 0.01381 g of $C_7H_6O_3$ found in the Assay for salicylic acid is equivalent to 0.01221 g of $C_7H_6O_2$.

For salicylic acid — Cool the titrated solution obtained in the Assay for benzoic acid, dilute to 250.0 ml with *water* and filter. To 5.0 ml of the filtrate add sufficient *iron(III) nitrate solution* to produce 50.0 ml. Filter, if necessary, to remove haze and measure the absorbance of the resulting solution at the maximum at about 530 nm (2.4.7) using *iron(III) nitrate solution* in the reference cell. Calculate the content of $C_7H_6O_3$ from the absorbance obtained by repeating the operation using 5 ml of a 0.024 per cent w/v solution of *salicylic acid* and beginning at the words 'add sufficient *iron(III) nitrate solution* .....'.

Storage. Store at a temperature not exceeding 30°.

Benzoic Acid Solution

Benzoic Acid Solution contains 50 g of benzoic acid, 750 ml of propylene glycol, diluted to 1000 ml with purified water.

Benzoic Acid Solution contains not less than 4.75 per cent w/v and not more than 5.25 per cent w/v of benzoic acid, $C_7H_6O_2$.

Category. Antifungal.

Identification

To 5 ml, add 30 ml of *1 M sulphuric acid* and extract the precipitated acid with three 25 ml quantities of *light petroleum ether (boiling range, 40° to 60°)*. Wash the combined extracts with three 25 ml quantities of *water*, filter through absorbent cotton and evaporate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzoic acid IPRS* or with the reference spectrum of benzoic acid.

B. Melting point (2.4.21). 121° ± 1°.

Tests

Weight per ml (2.4.29). 1.045 to 1.055 g.

Assay. To 10 ml, add 20 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01221 g of $C_7H_6O_2$

Labelling. The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

Benzoin

Benzoin is the balsamic resin obtained from *Styrax benzoin* Dryander or *Styrax parallelloneurus* Perkins, known in commerce as Sumatra Benzoin or from *Styrax tonkinensis* (Pierre) Craib ex Hartwich, or other species of the Section *Anthostyrax* of the genus *Styrax*, known in commerce as Siam Benzoin (Fam. Styraceae).

Benzoin contains not less than 25.0 per cent of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$, in Sumatra Benzoin and as benzoic acid, $C_7H_6O_2$, in Siam Benzoin, calculated on the dried basis.

Category. Topical protectant; expectorant and comforting by steam inhalation in acute laryngitis.

Description. *Unground Sumatra Benzoin* — Blocks or lumps of varying size, made up of tears compacted together, with a reddish-brown, reddish-grey or greyish-brown resinous mass, known in commerce as block benzoin. It also occurs in the form of tears with cream-coloured to yellowish surfaces; when fractured they exhibit milky-white surfaces.

Unground Siam Benzoin — Pebble-like tears of variable size and shape, compressed, yellowish-brown to rusty-brown externally, milky white on fracture, hard and brittle at ordinary temperatures but softened by heat.

Identification

A. To a solution in *ethanol (95 per cent)* add *water*; the solution becomes milky, and the mixture is acid to *litmus paper*.

B. Heat 0.5 g in a dry test-tube; it melts and evolves white fumes, which form a white needle-shaped crystalline sublimate.

C. Heat 0.5 g in a test-tube with 5 ml of *potassium permanganate solution*; a strong odour of benzaldehyde is obtained with Sumatra Benzoin.

D. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate*.

Test solution. Dissolve 2.0 g of the substance under examination in 100 ml of *ethanol (95 per cent)*.

Reference solution (a). A 0.05 per cent w/v solution of *benzoic acid IPRS* in *chloroform*.

Reference solution (b). A 0.05 per cent w/v solution of *cinnamic acid IPRS* in *chloroform*.

Reference solution (c). A 0.05 per cent w/v solution of *coniferyl benzoate IPRS* in *chloroform*.

Reference solution (d). A 0.05 per cent w/v solution of *cinnamoyl cinnamate IPRS* in *chloroform*.

Reference solution (e). A 0.05 per cent w/v solution of *propyl cinnamate IPRS* in *chloroform*.

Reference solution (f). A 0.05 per cent w/v solution of *cinnamoyl benzoate IPRS* in *chloroform*.

After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 5 minutes and examine under ultraviolet light at 254 nm. In the case of Sumatra Benzoin, the chromatogram obtained with the test solution exhibits four intense spots corresponding to spots in the chromatograms obtained with reference solution (b), (c), (d) and (e). In the case of Siam Benzoin, it exhibits intense spots corresponding to spots in the chromatograms obtained with reference solution (a), (c), (d) and (f).

Tests

Dammar gum. Determine by thin-layer chromatography (2.4.17), coating the plate with *aluminium oxide G*.

Mobile phase. A mixture of 60 volumes of *ether* and 40 volumes of *light petroleum* (80° to 100°).

Test solution. Dissolve by warming 0.2 g of the substance under examination in 10 ml of *ethanol (90 per cent)* and centrifuge.

Apply to the plate 5 µl of the test solution. Allow the mobile phase to rise 10 cm. Dry the plate in air, spray with *anisaldehyde-sulphuric acid reagent* and heat at 100° to 105° for 5 minutes. The chromatogram does not show any prominent spot with an R_f value between 0.4 and 1.0.

Foreign organic matter (2.6.1). Not more than 1.0 per cent.

Ethanol-soluble extractive. Not less than 75.0 per cent in Sumatra Benzoin and not less than 90.0 per cent in Siam Benzoin, determined by the following method. Weigh 2 g, in coarse powder, in a tared extraction thimble and insert the thimble in a Soxhlet or other suitable continuous extraction apparatus. Place 0.1 g of *sodium hydroxide* in the receiving flask of the apparatus, extract with *ethanol (95 per cent)* until extraction is complete (about 5 hours), dry the thimble to constant weight at 105° and calculate the ethanol-soluble extractive from the increase in weight of the thimble.

Acid-insoluble ash (2.3.19). Not more than 1.0 per cent in Sumatra Benzoin and not more than 0.5 per cent in Siam Benzoin, determined on 2.0 g.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 2.0 g, in coarse powder, by drying over *phosphorus pentoxide* at a pressure not exceeding 2.7 kPa for 4 hours.

Assay. Weigh 1.25 g and boil with 25 ml of *dilute ethanolic potassium hydroxide solution* under a reflux condenser for 1 hour. Remove the ethanol and digest the residue with 50 ml of hot *water* until diffused. Cool the liquid, add 150 ml of *water* and 1.5 g of *magnesium sulphate* dissolved in 50 ml of *water*. Mix thoroughly and set aside for 10 minutes. Filter, wash the residue on the filter with 20 ml of water, acidify the combined filtrate and washings with *hydrochloric acid* and extract with successive quantities of 50, 40, 30, 30 and 30 ml of *ether*. Combine the ether extracts and discard the aqueous portion. Extract with successive quantities of 20, 20, 10, 10 and 10 ml of *sodium bicarbonate solution*, washing each aqueous extract with the same 20 ml of *ether*. Discard the ether layers, acidify the combined aqueous extracts with *hydrochloric acid* and extract with successive quantities of 30, 20, 20 and 10 ml of *chloroform*, filtering each chloroform extract through a plug of cotton wool on which a layer of *anhydrous sodium sulphate* is placed. Evaporate the chloroform on a water-bath until about 10 ml remains and remove the remainder in a current of air stopping immediately when the last trace of solvent is removed. Dissolve the residue by warming with 10 ml of *ethanol (95 per cent)*, previously neutralised to *phenol red solution*, cool and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01482 g of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$, in Sumatra Benzoin and 0.01221 g of total balsamic acids, calculated as benzoic acid, $C_7H_6O_2$, in Siam Benzoin.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states whether the material is Sumatra Benzoin or Siam Benzoin.

Compound Benzoin Tincture

Friars' Balsam

Benzoin, in *moderately coarse powder*	100 g
Prepared Storax	75 g
Tolu Balsam	25 g
Aloes, in *moderately coarse powder*	20 g
Ethanol (90 per cent) sufficient to produce	1000 ml

Macerate the Benzoin, Prepared Storax, Tolu Balsam and Aloes with 800 ml of Ethanol (90 per cent) in a closed vessel for not less than 2 days with occasional shaking. Filter and pass sufficient Ethanol (90 per cent) through the filter to produce the required volume.

Compound Benzoin Tincture contains not less than 4.5 per cent w/v of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

Category. Topical protectant; expectorant and comforting by steam inhalation in acute laryngitis.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate*.

Test solution. Dilute 1 ml of the tincture with 4 ml of *ethanol (95 per cent)*. (The chromatographic profile may vary depending on the variety of Benzoin used).

Reference solution (a). A 0.05 per cent w/v solution of *benzoic acid IPRS* in *chloroform*.

Reference solution (b). A 0.05 per cent w/v solution of *cinnamic acid IPRS* in *chloroform*.

Reference solution (c). A 0,05 per cent w/v solution of *coniferyl benzoate IPRS* in *chloroform*.

Reference solution (d). A 0.05 per cent w/v solution of *cinnamoyl cinnamate IPRS* in *chloroform*.

Reference solution (e). A 0.05 per cent w/v solution of *propyl cinnamate IPRS* in *chloroform*.

Reference solution (f). A 0.05 per cent w/v solution of *cinnamoyl benzoate IPRS* in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 5 minutes and examine under ultraviolet light at 254 nm. In the case of Sumatra Benzoin, the chromatogram obtained with the test solution exhibits four intense spots corresponding to spots in the chromatograms obtained with reference solution (b), (c), (d) and (e). In the case of Siam Benzoin, it exhibits intense spots corresponding to spots in the chromatograms obtained with reference solution (a), (c), (d) and (f).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate*.

Test solution. Dilute 1 ml of the *tincture* with 4 ml of *ethanol (95 per cent)*.

Reference solution. A 0.05 per cent w/v solution of *styrene IPRS* in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 5 minutes and examine under ultraviolet light at 254 nm. The chromatogram obtained with the test solution exhibits an intense spot corresponding to the spot in the chromatogram obtained with the reference solution (Prepared Storax).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate*.

Test solution. Dilute 1 ml of the *tincture* with 4 ml of *ethanol (95 per cent)*.

Reference solution (a). A 0.05 per cent w/v solution of *benzoyl benzoate IPRS* in *chloroform*.

Reference solution (b). A 0.05 per cent w/v solution of *benzoyl cinnamate IPRS* in *chloroform*.

Reference solution (c). A 0.05 per cent w/v solution of *eugenol IPRS* in *chloroform*.

Reference solution (d). A 0.05 per cent w/v solution of *vanillin IPRS* in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 5 minutes and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution exhibits spots corresponding to the spots in the chromatogram obtained with reference solutions, (a), (b), (c) and (d) (Tolu Balsam).

D. Carry out the method for thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *ethyl acetate*, 13.5 volumes of *methanol* and 10 volumes of *water*.

Test solution. Dilute 1 ml of the *tincture* with 4 ml of *ethanol (95 per cent)*.

Reference solution. A 0.5 per cent w/v solution of *barbaloin IPRS* in *methanol*.

Apply to the plate 50 µl of each solution as bands 20 mm long and not more than 3 mm wide. Allow the mobile phase to rise 10 cm. Dry the plate in air until the odour of the solvent is no longer detectable, spray with a 10 per cent w/v solution of *potassium hydroxide* in *methanol* and examine in ultraviolet light at 365 nm. The chromatogram obtained with the test solution exhibits a yellow fluorescent band corresponding to the band obtained in the chromatogram obtained with the reference solution and a light blue fluorescent band with a lower R_f value due to aloesine. Heat the plate at 110° for 5 minutes; a violet fluorescent band just below the band corresponding to barbaloin may also be seen in the chromatogram obtained with the test solution (Aloes).

Tests

Weight per ml (2.4.29). 0.870 g to 0.885 g.

Ethanol content. 70.0 to 77.0 per cent v/v, determined by Method II (2.3.45).

Total solids. Not less than 13.5 per cent w/v, determined on 1 ml by drying in an oven at 105° for 4 hours.

Assay. Evaporate 10 ml to a thick consistency on a water-bath. Boil the residue with 25 ml of *ethanolic potassium hydroxide solution* under a reflux condenser for 1 hour. Remove the ethanol and digest the residue with 50 ml of hot *water* until diffused. Cool the liquid, add 150 ml of *water* and 1.5 g of *magnesium sulphate* dissolved in 50 ml of *water*. Mix thoroughly and set aside for 10 minutes. Filter, wash the residue on the filter with 20 ml of water, acidify the combined filtrate and washings with *hydrochloric acid* and extract with successive quantities of 50, 40, 30, 30 and 30 ml of *ether*. Combine the ether extracts and discard the aqueous portion. Extract with successive quantities of 20, 20, 10, 10 and 10 ml of *sodium bicarbonate solution*, washing each aqueous extract with the same 20 ml of *ether*. Discard the ether layers, acidify the combined aqueous extracts with *hydrochloric acid* and extract with successive quantities of 30, 20, 20 and 10 ml of *chloroform*, filtering each chloroform extract through a plug of cotton wool on which a layer of *anhydrous sodium sulphate* is placed. Evaporate the chloroform on a water-bath until about

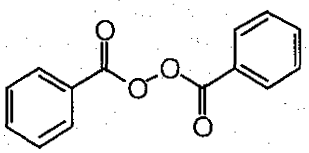
10 ml remains and remove the remainder in a current of air stopping immediately when the last trace of solvent is removed. Dissolve the residue by warming with 10 ml of *ethanol (95 per cent)*, previously neutralised to *phenol red solution*, cool and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01482 g of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

Storage. Store protected from light in tightly-closed containers and avoid exposure to direct sunlight and to excessive heat.

Labelling. The label states that it is flammable.

Hydrous Benzoyl Peroxide



$C_{14}H_{10}O_4$ Mol. Wt. 242.2

Hydrous Benzoyl Peroxide is dibenzoyl peroxide.

Hydrous Benzoyl Peroxide contains not less than 70.0 per cent and not more than 77.0 per cent of $C_{14}H_{10}O_4$.

Category. Antiacne.

Description. A white or almost white, granular or amorphous powder.

Identification

NOTE — It loses water rapidly on exposure to air with a risk of explosion. Mix the entire sample thoroughly before carrying out the following tests.

Test A, C and D may be omitted if B is carried out.

A. When examined in the range of 250 nm to 300 nm (2.4.7), a 0.008 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maxima at about 274 nm and shoulder at about 282 nm and when examined in the range of 220 nm to 250 nm (2.4.7), a 0.0008 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maxima at about 235 nm.

B. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzoyl peroxide IPRS* or with the reference spectrum of benzoyl peroxide.

C. Weigh 25 mg, dissolve in 2 ml of *acetone*, add 1 ml of 1.0 per cent solution of *diethylphenylenediamine sulphate* and mix; a red colour develops which quickly darkens and becomes dark violet within 5 minutes. On heating, a gas is evolved and the solution becomes red.

D. To 1 g add 5 ml of *ethanol (96 per cent)*, 5 ml of *dilute sodium hydroxide solution* and 10 ml of *water*. Boil the mixture under reflux for 20 minutes and cool. The solution gives reaction (C) of benzoates (2.3.1).

Tests

Acidity. Dissolve a quantity of the substance under examination containing 1 g benzoyl peroxide in 25 ml of *acetone*, add 75 ml of *water* and filter. Wash the residue with two quantities, each of 10 ml of *water*. Combine the filtrate and the washings and add 0.25 ml of *phenolphthalein solution* as indicator. Titrate with *0.1 M sodium hydroxide* to a pink colour; not more than 1.25 ml of *0.1 M sodium hydroxide* is required for neutralisation. Carry out a blank test.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve a quantity of the substance under examination containing 0.1 g benzoyl peroxide in *acetonitrile* and dilute to 50.0 ml with *acetonitrile*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*. Further dilute 1.0 ml of the solution to 10.0 ml with *acetonitrile*.

Reference solution (b). A 0.003 per cent w/v solution of *benzoic acid* in the mobile phase.

Reference solution (c). A 0.0005 per cent w/v solution of *ethyl benzoate* in the mobile phase.

Reference solution (d). A 0.0005 per cent w/v solution of *benzaldehyde* in the mobile phase.

Reference solution (e). Dissolve 30 mg each of *benzoic acid* and *benzaldehyde* in the mobile phase and dilute to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 0.1 ml of *glacial acetic acid*, 50 volumes of *water* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Name	Relative retention time
Benzoic acid	0.15
Benzaldehyde	0.2
Ethyl benzoate	0.4
Benzoyl peroxide (Retention time: about 28.4 minutes)	1.0

Inject reference solution (e). The test is not valid unless the resolution between the peaks corresponding to benzoic acid and benzaldehyde is not less than 6.0.

Inject reference solution (a), (b), (c), (d) and the test solution. Run the chromatogram twice the retention time of the principal peak for test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to benzaldehyde is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.25 per cent), the area of peak corresponding to benzoic acid is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent), the area of peak corresponding to ethyl benzoate is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.25 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Chlorides (2.3.12). Dissolve a quantity of the substance under examination containing 0.5 g of benzoyl peroxide in 15 ml of *acetone,* add, while stirring, 50 ml of *0.05 M nitric acid.* Allow to stand for 10 minutes and filter. Wash the residue with 2 quantities, each of 10 ml, of *0.05 M nitric acid.* Combine the filtrate and the washings and dilute to 100 ml with *0.05 M nitric acid.* Dilute 12.5 ml of the solution to 15.0 ml with *water.* The solution complies with the limit test for chlorides (0.4 per cent).

Water (2.3.43). Not less than 20.0 per cent, determined on 5.0 ml of 2.5 percent w/v solution of sample in *dimethylformamide* and using a mixture of 20.0 ml of *anhydrous methanol* and 3.0 ml of 10.0 per cent w/v solution of *potassium iodide* in *dimethylformamide* in titration vessel instead of *methanol.*

Assay. Dissolve 2.5 g of substance under examination immediately before use in 75 ml of *dimethylformamide* and dilute to 100.0 ml with the *dimethylformamide.*

To 5.0 ml of the solution, add 20 ml of *acetone* and 3 ml of *potassium iodide solution* prepared by dissolving 500 g of *potassium iodide* in 1000 ml of *water.* Mix and allow to stand for 1 minute. Titrate with *0.1 M sodium thiosulphate*, using 1 ml of *starch solution* added towards the end of the titration as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.01211 g of $C_{14}H_{10}O_4$.

Storage. Store protected from light in a container that has been treated to reduce static discharge and that has a device for release of excess pressure, at a temperature between 2° to 8°.

Benzoyl Peroxide Cream

Benzoyl Peroxide Cream contains Hydrous Benzoyl Peroxide in a suitable basis.

Benzoyl Peroxide Cream contains not less than 90.0 per cent and not more than 110.0 per cent of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$.

Usual strength. 10 per cent w/w.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica GF254.*

Mobile phase. A mixture of 1 volume of *glacial acetic acid,* 2 volumes of *dichloromethane* and 50 volumes of *toluene.*

Test solution. Disperse a quantity of the cream containing 50 mg of anhydrous Benzoyl Peroxide with 10 ml of *chloroform* and filter.

Reference solution. A 0.5 per cent w/v solution of *benzoyl peroxide* in *chloroform.*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the cream containing 0.1 g of anhydrous Benzoyl Peroxide in 25 ml of *acetonitrile* and dilute to 50.0 ml with *water,* filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). A 0.02 per cent w/v solution of *benzoic acid* in the mobile phase.

Reference solution (c). A 0.002 per cent w/v solution of *ethyl benzoate* in the mobile phase.

Reference solution (d). A 0.002 per cent w/v solution of *benzaldehyde* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 1 volume of *glacial acetic acid,* 500 volumes of *acetonitrile* and 500 volumes of *water,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Inject reference solution (a), (b), (c), (d) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to benzoic acid is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (10.0 per cent), the area of any peak corresponding to ethyl benzoate is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any peak corresponding to benzaldehyde is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Other tests. Comply with the tests stated under Creams.

Assay. Disperse a quantity of the cream containing 0.25 g of anhydrous Benzoyl Peroxide with 50 ml of *acetone* and dilute to 100.0 ml with *acetone*. To 10 ml, add 25 ml of a 20 per cent w/v solution of *potassium iodide*, mix, stopper the flask and allow to stand for 15 minutes protected from light. Add 25 ml of *acetone* and titrate with *0.01 M sodium thiosulphate* using *starch mucilage* as indicator added towards the end of the titration. Repeat the operation without the cream. The difference between the titrations represents the amount of *sodium thiosulphate* required.

1 ml of *0.01 M sodium thiosulphate* is equivalent to 0.001211 g of $C_{14}H_{10}O_4$.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

Benzoyl Peroxide Gel

Benzoyl Peroxide Gel is a solution of Hydrous Benzoyl Peroxide in a suitable water- soluble basis.

Benzoyl Peroxide Gel contains not less than 90.0 per cent and not more than 110.0 per cent of anhydrous benzoyl peroxide, $C_{14}H_{10}O_4$.

Usual strengths. 2.5 per cent w/v; 5 per cent w/v; 10 per cent w/v.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *glacial acetic acid*, 2 volumes of *dichloromethane* and 50 volumes of *toluene*.

Test solution. Disperse a quantity of the gel containing 50 mg of anhydrous Benzoyl Peroxide with 10 ml of *chloroform* and filter.

Reference solution. A 0.5 per cent w/v solution of *benzoyl peroxide* in *chloroform*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the gel containing 0.1 g of anhydrous Benzoyl Peroxide in 25 ml of *acetonitrile* and dilute to 50.0 ml with *water*, filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). A 0.02 per cent w/v solution of *benzoic acid* in the mobile phase.

Reference solution (c). A 0.002 per cent w/v solution of *ethyl benzoate* in the mobile phase.

Reference solution (d). A 0.002 per cent w/v solution of *benzaldehyde* in the mobile phase.

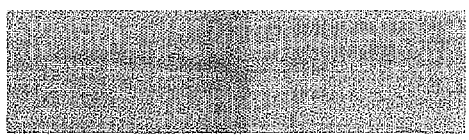
Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 0.1 ml of *glacial acetic acid*, 50 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Inject reference solution (a), (b), (c), (d) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to benzoic acid is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (10.0 per cent), the area of any peak corresponding to ethyl benzoate is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any peak corresponding to benzaldehyde is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Other tests. Comply with the tests stated under Gels.

Assay. Disperse a quantity of the gel containing 0.25 g of anhydrous Benzoyl Peroxide with 50 ml of *acetone* and dilute to 100.0 ml with *acetone*. To 10 ml, add 25 ml of a 20 per cent w/v solution of *potassium iodide*, mix, stopper the flask and allow to stand for 15 minutes protected from light. Add 25 ml

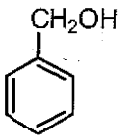


of *acetone* and titrate with *0.01 M sodium thiosulphate* using *starch mucilage* as indicator added towards the end of titration. Repeat the operation without gel. The difference between the titrations represents the amount of sodium thiosulphate required.

1 ml of *0.01 M sodium thiosulphate* is equivalent to 0.001211 g of $C_{14}H_{10}O_4$.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous benzoyl peroxide.

Benzyl Alcohol



C_7H_8O Mol. Wt. 108.1

Benzyl Alcohol contains not less than 98.0 per cent and not more than 100.5 per cent of C_7H_8O.

Category. Local anaesthetic; disinfectant.

Description. Clear, colourless, oily liquid.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzyl alcohol IPRS* or with the reference spectrum of benzyl alcohol.

Tests

Appearance of solution. A 1.0 per cent v/v solution is clear (2.4.1), colourless and oily liquid (2.4.1).

Peroxide value (2.3.35). Not more than 5.0.

Wt. per ml (2.4.29). 1.04 g to 1.05 g.

Refractive index (2.4.27). 1.536 to 1.542.

Acid Value (2.3.23). Not more than 0.5.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. The substance under examination.

Reference solution (a). Dissolve 0.1 g of *ethylbenzene* in 10.0 ml of the test solution. Dilute 2.0 ml of the solution to 20.0 ml with the test solution.

Reference solution (b). Dissolve 2.0 g of *dicyclohexyl* in 10.0 ml of the test solution. Dilute 2.0 ml of the solution to 20.0 ml with the test solution.

Reference solution (c). Dissolve 0.75 g of *benzyl alcohol impurity A (benzaldehyde)* and 0.5 g of *benzyl alcohol impurity B (cyclohexyl-methanol)* in 25.0 ml of the test solution. Add 1.0 ml of the solution to a mixture of 2.0 ml of reference solution (a) and 3.0 ml of reference solution (b) and dilute to 20.0 ml with the test solution.

Reference solution (d). Dissolve 0.25 g of *benzaldehyde* and 0.5 g of *cyclohexylmethanol* in 25.0 ml of the test solution. Add 1.0 ml of the solution to a mixture of 2.0 ml of reference solution (a) and 2.0 ml of reference solution (b) and dilute to 20.0 ml with the test solution.

Chromatographic system

- a capillary column 30 m x 0.32 mm, packed with fused silica coated with macrogol 20000 (film thickness 0.5 µm),
- temperature:

column	time (min.)	temperature (°)
	0-34	50-220
	34-69	220

- inlet port: 200° and detector. 310°,
- flame ionization detector,
- linear velocity: 25 cm/second, using nitrogen as the carrier gas,

NOTE — Benzyl alcohol not intended for parenteral use.

Inject 0.1 µl reference solution (c). The relative retention time with reference to benzyl alcohol for ethyl benzene is about 0.28, for dicyclohexyl is about 0.59, for benzyl alcohol impurity A is about 0.68 and for benzyl alcohol impurity B is about 0.71. The test is not valid unless the resolution between the peaks due to benzyl alcohol impurity A and benzyl alcohol impurity B is not less than 3.0.

If any peaks in the chromatogram obtained with the test solution have the same retention time as the peaks due to ethyl benzene or dicyclohexyl, subtract the areas of any such peaks from the peak areas at these retention times in the chromatograms obtained with reference solution (c) or (d) (corrected peak areas of ethyl benzene and dicyclohexyl). Any such peaks in the chromatogram obtained with the test solution are to be included in the assessments for the sum of other peaks.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak corresponding to benzyl alcohol impurity A is not more than the area of the peak due to benzyl alcohol impurity A in the chromatogram obtained with reference solution (c) (0.15 per cent). The area of secondary peak corresponding to benzyl alcohol impurity B is not more than the area of the peak due to benzyl alcohol impurity B in the chromatogram obtained with reference solution (c) (0.1 per

cent). The sum of the areas of all other secondary peaks with relative retention time less than benzyl alcohol is not more than 4 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (c) (0.04 per cent). The sum of the areas of all other secondary peaks with relative retention time more than benzyl alcohol is not more than the area of the peak due to dicyclohexyl in the chromatogram obtained with reference solution (c) (0.3 per cent). Ignore any peak with an area less than 0.01 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (c) (0.0001 per cent).

NOTE — Benzyl alcohol intended for parenteral use.

Inject 0.1 µl reference solution (d). The relative retention time with reference to benzyl alcohol for ethyl benzene is about 0.28, for dicyclohexyl is about 0.59, for benzyl alcohol impurity A is about 0.68 and for benzyl alcohol impurity B is about 0.71. The test is not valid unless the resolution between the peaks due to benzyl alcohol impurity A and benzyl alcohol impurity B is not less than 3.0.

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak corresponding to benzyl alcohol impurity A is not more than the area of the peak due to benzyl alcohol impurity A in the chromatogram obtained with reference solution (d) (0.05 per cent). The area of secondary peak corresponding to benzyl alcohol impurity B is not more than the area of the peak due to benzyl alcohol impurity B in the chromatogram obtained with reference solution (d) (0.1 per cent). The sum of the areas of all other secondary peaks with relative retention time less than benzyl alcohol is not more than twice the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (d) (0.02 per cent). The sum of the areas of all other secondary peaks with relative retention time more than benzyl alcohol is not more than the area of the peak due to dicyclohexyl in the chromatogram obtained with reference solution (d) (0.2 per cent). Ignore any peak with an area less than 0.01 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (d) (0.0001 per cent).

Residue on evaporation. Not more than 0.05 per cent.

After ensuring that the substance under examination complies with the test for peroxide value, evaporate 10.0 g of Benzyl Alcohol, on a hot plate at a temperature not exceeding 200°. Ensure that the substance under examination does not boil during examination and dry the residue at 200° for 1 hour. Cool in a desiccator and weigh.

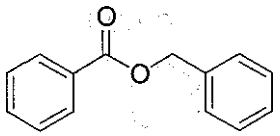
Assay. To 1.5 g add *25* ml of a mixture of 1 volume of *acetic anhydride* and 7 volumes of *pyridine* and heat on a water-bath for thirty minutes. Cool, add 25 ml of *water*, and titrate with *1 M sodium hydroxide*, using *phenolphthalein solution* as indicator. Repeat the operation without the substance under examination; the difference between the titrations represents the amount of alkali required by the benzyl alcohol.

1 ml of *1 M sodium hydroxide* is equivalent to 0.1081 g of C_7H_8O.

Storage. Store protected from light and moisture.

Labelling. The label states, where appropriate, the contents are intended for use in the manufacture of parenteral preparations.

Benzyl Benzoate



$C_{14}H_{12}O_2$ Mol. Wt. 212.2

Benzyl Benzoate is the benzyl ester of benzoic acid.

Benzyl Benzoate contains not less than 99.0 per cent and not more than 100.5 per cent w/w of $C_{14}H_{12}O_2$.

Category. Anti-parasitic (for topical treatment of scabies).

Description. Colourless crystals or a clear, colourless, oily liquid.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzyl benzoate IPRS* or with the reference spectrum of benzyl benzoate.

Tests

Congealing temperature (2.4.10). Not below 17.0°.

Relative density (2.4.29). 1.113 to 1.118.

Refractive index (2.4.27). 1.568 to 1.570.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Boil a convenient quantity of *ethanol (95 per cent)* thoroughly to expel carbon dioxide and neutralise to *phenolphthalein solution*. Weigh 2 g of the substance under examination, dissolve in 5 ml of the neutralised ethanol contained in a hard-glass flask and neutralise the free acid in the solution with *0.5 M ethanolic potassium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Add 40 ml of *0.5 M ethanolic potassium hydroxide* and boil under a reflux condenser on a water-bath for 1 hour. Add 20 ml of *water* and titrate the excess of alkali with *0.5 M hydrochloric*

acid using a further 0.2 ml of *phenolphthalein solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the alkali required to saponify the benzyl benzoate.

1 ml of *0.5 M ethanolic potassium hydroxide* is equivalent to 0.1061 g of $C_{14}H_{12}O_2$.

Storage. Store protected from light and air in well-filled containers.

Benzyl Benzoate Application

Benzyl Benzoate Application contains 25 per cent w/w of Benzyl Benzoate in a suitable oil-in-water emulsified base.

Benzyl Benzoate Application contains not less than 22.5 per cent and not more than 27.5 per cent w/w of benzyl benzoate, $C_{14}H_{12}O_2$.

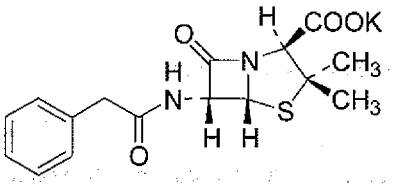
Assay. Dissolve 8.0 g in 10 ml of *ethanol (95 per cent)* previously neutralised with *0.1 M sodium hydroxide* contained in a hard-glass flask and neutralise the free acid in the solution with *0.5 M ethanolic potassium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Add 40 ml of *0.5 M ethanolic potassium hydroxide* and boil under a reflux condenser on a water-bath for 1 hour. Add 20 ml of *water* and titrate the excess of alkali with *0.5 M hydrochloric acid* using a further 0.2 ml of *phenolphthalein solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the alkali required to saponify the benzyl benzoate.

1 ml of *0.5 M ethanolic potassium hydroxide* is equivalent to 0.1061 g of $C_{14}H_{12}O_2$.

Labelling. The label states that the contents should be shaken before use.

Benzylpenicillin Potassium

Penicillin G Potassium



$C_{16}H_{17}KN_2O_4S$ Mol. Wt. 372.5

Benzylpenicillin Potassium is potassium (6*R*)-6-(2- phenylacetamido)penicillanate, produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Benzylpenicillin Potassium contains not less than 96.0 per cent and not more than 100.5 per cent of penicillins, calculated as $C_{16}H_{17}KN_2O_4S$ on the dried basis.

Category. Antibacterial.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzylpenicillin potassium IPRS* or with the reference spectrum of benzylpenicillin potassium.

B. It gives reaction (A) of potassium salts (2.3.1).

Tests

pH (2.4.24). 5.5 to 7.5, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). + 270.0° to + 300.0°, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water.*

Light absorption (2.4.7). Dissolve 94 mg in sufficient *water* to produce 50.0 ml. Measure the absorbance of the solution at about 325 nm, at about 280 nm and at the maximum at about 264 nm, diluting the solution, if necessary, for the measurement at the maximum at about 264 nm. Absorbances at about 325 nm and 280 nm, not more than 0.10 and that at the maximum at about 264 nm, calculated on the basis of the undiluted solution (0.188 per cent w/v), 0.80 to 0.88.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay.

Inject reference solution (d) and elute isocratically using the chosen mobile phase. Inject test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following gradient:

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
20	0	100
35	0	100
50	70	30

Inject water and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution (a). Dissolve 50 mg of the substance under examination in *water* and dilute to 50.0 ml with the same solvent.

Test solution (b). Dissolve 80 mg of the substance under examination in *water* and dilute to 20.0 ml with the same solvent.

Reference solution (a). Dissolve 50 mg of *benzylpenicillin potassium IPRS* in *water* and dilute to 50.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of *benzylpenicillin potassium IPRS* and 10 mg of *phenylacetic acid IPRS* in *water* and dilute to 50.0 ml with the same solvent.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with *water*. Dilute 1.0 ml of the solution to 50.0 ml with the same solvent.

Reference solution (d). Dilute 4.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute orthophosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,

  B. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute orthophosphoric acid*, 40 volumes of *water* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Equilibrate the column with a mobile phase ratio A:B of 70:30.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the capacity factor for the second peak (benzylpenicillin) is 4.0 to 6.0.

Inject reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

Inject test solution (a) and reference solution (a).

Calculate the content of benzylpenicillin potassium.

Benzylpenicillin Potassium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.16 Endotoxin Unit per mg.

Benzylpenicillin Potassium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

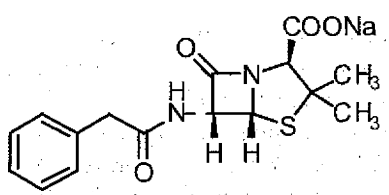
Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Benzylpenicillin Sodium

Penicillin G Sodium



$C_{16}H_{17}N_2NaO_4S$ Mol. Wt. 356.4

Benzylpenicillin Sodium is sodium (6*R*)-6-(2-phenylacetamido) penicillanate, produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Benzylpenicillin Sodium contains not less than 96.0 per cent and not more than 100.5 per cent of penicillins, calculated as $C_{16}H_{17}N_2NaO_4S$ on the dried basis.

Category. Antibacterial.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzylpenicillin sodium IPRS* or with the reference spectrum of benzylpenicillin sodium.

B. It gives reaction (A) of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.5 to 7.5, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +285° to +310°, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water*.

Light absorption (2.4.7). Dissolve 90 mg in sufficient *water* to produce 50.0 ml. Measure the absorbance of the solution at about 325 nm, at about 280 nm and at the maximum at about

264 nm, diluting the solution, if necessary, for the measurement at about 264 nm. Absorbances at about 325 nm and 280 nm, not more than 0.10 and that at the maximum at about 264 nm, calculated on the basis of the undiluted solution (0.18 per cent w/v), 0.80 to 0.88.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay.

Inject reference solution (d) and elute isocratically using the chosen mobile phase. Inject test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following gradient:

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
20	0	100
35	0	100
50	70	30

Inject water and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b) the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography, (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution (a). Dissolve 50.0 mg of the substance under examination in *water* and dilute to 50.0 ml with *water*.

Test solution (b). Dissolve 80.0 mg of the substance under examination in *water* and dilute to 20.0 ml with *water*.

Reference solution (a). Dissolve 50.0 mg of *benzylpenicillin sodium IPRS* in *water* and dilute to 50.0 ml with *water*.

Reference solution (b). Dissolve 10 mg of *benzylpenicillin sodium IPRS* and 10 mg of *phenylacetic acid IPRS* in *water* and dilute to 50.0 ml with *water*.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with *water*. Dilute 1.0 ml of the solution to 50.0 ml with *water*.

Reference solution (d). Dilute 4.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system

- a stainless steel a column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute orthophosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,

  B. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen* phosphate adjusted to pH 3.5 with a 500 g per litre solution of *dilute orthophosphoric acid*, 40 volumes of *water* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Equilibrate the column with a mobile phase ratio A:B of 70:30.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the capacity factor for the second peak (benzylpenicillin) is 4.0 to 6.0.

Inject reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

Inject reference solution (a) and test solution (a).

Calculate the content of $C_{16}H_{17}N_2NaO_4S$.

Benzylpenicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.16 Endotoxin Unit per mg.

Benzylpenicillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Benzylpenicillin Injection

Penicillin G Injection

Benzylpenicillin Injection is a sterile material consisting of Benzylpenicillin Potassium or Benzylpenicillin Sodium with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Usual strengths. The equivalent of 150 mg (250,000 Units), 300 mg (500,000 Units) and 600 mg (1,000,000 Units) of Benzylpenicillin.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Benzylpenicillin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of penicillins, calculated as $C_{16}H_{18}N_2O_4S$.

Description. A white or almost white crystalline powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *benzylpenicillin potassium IPRS* or with the reference spectrum of benzylpenicillin potassium.

or

Compare the spectrum with that obtained with *benzylpenicillin sodium IPRS* or with the reference spectrum of benzylpenicillin sodium.

B. It gives reaction (A) of potassium salts or sodium salts (2.3.1).

Tests

pH (2.4.24). 5.5 to 7.5, determined in a 10.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay.

Inject reference solution (d) and elute isocratically using the chosen mobile phase. Inject test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following gradient:

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
20	0	100
35	0	100
50	70	30

Inject reference solution (d) and test solution (b). In the chromatogram obtained with test solution (b), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.16 Endotoxin Unit per mg.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography, (2.4.14).

NOTE—Prepare the solutions immediately before use.

Determine the weight of the contents of 10 containers.

Test solution (a). Dissolve 50 mg of the mixed contents of the 10 containers in *water* and dilute to 50.0 ml with *water*.

Test solution (b). Dissolve 80 mg of the substance under examination in *water* and dilute to 20.0 ml with *water*.

Reference solution (a). Dissolve 50 mg of *benzylpenicillin sodium IPRS* in *water* and dilute to 50.0 ml with *water*.

Reference solution (b). Dissolve 10 mg of *benzylpenicillin sodium IPRS* and 10 mg of *phenylacetic acid IPRS* in *water* and dilute to 50.0 ml with *water*.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with *water*. Dilute 1.0 ml of the solution to 50.0 ml with *water*.

Reference solution (d). Dilute 4.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute orthophosphoric acid*, 30 volumes of *methanol* and 60 volumes of *water*,

  B. a mixture of 10 volumes of a 68 g per litre solution of *potassium dihydrogen phosphate* adjusted to pH 3.5 with a 500 g per litre solution of *dilute orthophosphoric acid*, 40 volumes of *water* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Equilibrate the column with a mobile phase ratio A:B of 70:30.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the capacity factor for the second peak (benzylpenicillin) is 4.0 to 6.0.

Inject reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

Inject reference solution (a) and test solution (a).

Calculate the content of benzylpenicillin sodium in the injection.

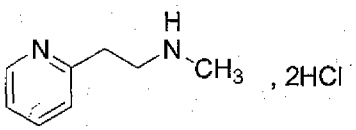
1 mg of $C_{16}H_{17}N_2NaO_4S$ is equivalent to 0.9383 mg of $C_{16}H_{18}N_2O_4S$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) whether the contents are Benzylpenicillin Potassium or Benzylpenicillin Sodium; (2) the name of any added buffering agents.

Betahistine Hydrochloride

Betahistine Dihydrochloride



$C_8H_{12}N_2, 2HCl$ Mol. Wt. 209.1

Betahistine Hydrochloride is *N*-methyl-2-(2-pyridyl) ethylamine dihydrochloride.

Betahistine Hydrochloride contains not less than 98.5 per cent and not more than 102.0 per cent of $C_8H_{12}N_2, 2HCl$, calculated on the dried basis.

Category. Antihistaminic.

Description. A white to off-white, crystalline powder; sometimes clumped, odourless or almost odourless, very hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betahistine hydrochloride IPRS* or with the reference spectrum of betahistine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives the reaction (A) of chlorides (2.3.12).

Tests

Appearance of solution. A 10 per cent w/v solution in *water* is clear (2.4.1) and not more intensely coloured than reference solution BS8 (2.4.1).

pH (2.4.24). 2.0 to 3.0, determined in a 10 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dissolve 10 mg of *betahistine dihydrochloride IPRS* and 10 mg of *2-vinylpyridine* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 2.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 2 g of *sodium lauryl sulphate* in a mixture of 15 ml of a 10.0 per cent v/v solution of *sulphuric acid*, 35 ml of a 1.7 per cent w/v solution of *tetrabutylammonium hydrogen sulphate* and 650 ml of *water*; adjusted to pH 3.3 using *dilute sodium hydroxide solution* and mix with 300 ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Betahistine impurity B[1]	0.2	0.4
Betahistine impurity A[2]	0.3	—
Betahistine (Retention time: about 7 minutes)	1.0	—
Betahistine impurity C[3]	3.0	—

[1]2-(pyridin-2-yl)ethanol,

[2]2-vinylpyridine,

[3]*N*-methyl-2-(pyridin-2-yl)-*N*-[2-(pyridin-2-yl)ethyl]ethanamine.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to 2-vinylpyridine and betahistine is not less than 3.5.

Inject reference solution (b), (c) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to betahistine impurity A, betahistine impurity B and betahistine impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.04 per cent w/v solution of *betahistine dihydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.0 mm packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: dissolve 0.45 g *ammonium acetate* and 0.4 ml *glacial acetic acid* in 650 ml of *water*, add 350 ml of *acetonitrile* and add 2.88 g of *sodium laurylsulphate* and mix,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{12}N_2$, 2HCl.

Storage. Store protected from light.

Betahistine Tablets

Betahistine Hydrochloride Tablets

Betahistine Tablets contain Betahistine Dihydrochloride.

Betahistine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of betahistine dihydrochloride, $C_8H_{12}N_2$,2HCl.

Usual strengths. 8 mg; 16 mg.

Identification

Dissolve the powdered tablet containing about 0.1 g of Betahistine Hydrochloride in 5 ml of *water*, add 0.5 ml of *5 M sodium hydroxide*, extract with 5 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate* with 2 ml of *dichloromethane* and evaporate the solution to dryness. The residue complies with the following test.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betahistine hydrochloride IPRS* treated in the same manner or with the reference spectrum of betahistine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of buffer solution prepared by dissolving 21.9 g of *anhydrous disodium hydrogen orthophosphate* and 4.83 g of *citric acid* in 1000 ml of *water*, adjusted to pH 6.8 with *1 M sodium hydroxide*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 256 nm (2.4.7). Calculate the content of $C_8H_{12}N_2$, 2HCl in the medium from the absorbance obtained from a solution of known concentration of *betahistine hydrochloride IPRS* in the same medium.

Q. Not less than 80 per cent of the stated amount of $C_8H_{12}N_2$,2HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 32 mg of Betahistine Dihydrochloride in 50 ml of mobile phase and dilute to 100.0 ml with mobile phase, centrifuge and use the supernatant liquid.

Reference solution (a). Dilute 1.0 ml of the test solution to 500.0 ml with the mobile phase.

Reference solution (b). A 0.00064 per cent w/v solution of *betahistine impurity C IPRS (N-methyl-2-(pyridin-2-yl)-N-[2-(pyridine-2- yl)ethyl]ethanamine trihydrochloride)* in the mobile phase.

Reference solution (c). A 0.000032 per cent w/v solution of *betahistine impurity A IPRS(2-vinylpyridine)* in *acetonitrile*.

Reference solution (d). A solution containing 0.00064 per cent w/v each of *betahistine impurity C IPRS* and *betahistine dihydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Zorbax XDB Eclipse),
- mobile phase: dissolve 0.4 g of *hexylamine* in 600 ml of a solution containing 0.46 per cent w/v solution of *sodium dihydrogen orthophosphate monohydrate* and

0.27 per cent w/v of *sodium lauryl sulphate*, add 400 ml of *acetonitrile*, mix and adjusted to pH 3.5 using *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to N-methyl-2-(pyridin-2-yl)-N-[2-(pyridine-2- yl)ethyl]ethanamine trihydrochloride and betahistine dihydrochloride is not less than 3.0.

Inject reference solution (a), (b), (c) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to betahistine impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and the area of any peak corresponding to betahistine impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Uniformity of content. Complies with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

Test solution. Disperse one tablet to a 25-ml volumetric flask with about 15 ml of mobile phase, mix with the aid of ultrasound and dilute to 25.0 ml with the mobile phase, filter. Dilute with mobile phase to achieve concentration of 0.032 per cent w/v of betashistine hydrochloride.

Calculate the content of $C_8H_{12}N_2$,2HCl in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powdered tablet containing 32 mg of Betahistine Dihydrochloride, disperse in 50 ml of mobile phase and dilute to 100.0 ml with mobile phase and filter.

Reference solution. A 0.032 per cent w/v solution of *betahistine hydrochloride IPRS* in mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: dissolve 2.76 g of *sodium dihydrogen phosphate monohydrate* and 1.6 g of *sodium dodecyl-sulphate* in 600 ml of *water*, add 0.4 g of *hexylamine* and 400 ml of *acetonitrile*, adjusted to pH 3.5 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

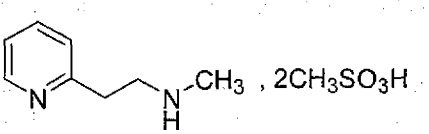
Inject the reference solution and the test solution.

Calculate the content of $C_8H_{12}N_2$, 2HCl in the tablet.

Storage. Store protected from light.

Betahistine Mesylate

Betahistine Mesilate



$C_8H_{12}N_2$,2CH_3SO_3H Mol Wt. 328.4

Betahistine Mesilate is 2-[(2-Methylamino)ethyl]pyridine bis(methanesulphonate).

Betahistine Mesylate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_8H_{12}N_2$, 2CH_3SO_3H, calculated on anhydrous basis.

Category. Antivertigo.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betahistine mesylate IPRS* or with the reference spectrum of betahistine mesylate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 0.75 volume of *ammonia*, 15 volumes of *ethyl acetate* and 30 volumes of *methanol*.

Test solution. Dissolve 10 mg of the substance under examination in *ethanol (95 per cent)* and dilute to 2.0 ml with *ethanol (95 per cent)*.

Reference solution. A 0.5 per cent w/v solution of *betahistine mesylate IPRS* in *ethanol (95 per cent)*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and heat at 110° for 10 minutes, allow to cool and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. To 0.1 g, add 5 ml of *dilute hydrochloric acid* and shake for about 5 minutes. Add 1 ml of *barium chloride solution*. The solution remains clear. To a further 0.1 g, add 0.5 g of *anhydrous sodium carbonate*, mix and ignite until a white residue is obtained. Allow to cool and dissolve the residue in 7 ml of *water*. It gives reaction (A) of sulphates (2.3.1).

Tests

Appearance of solution. A 10 per cent w/v solution in *carbon dioxide-free water* (Solution A) is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 2.0 to 3.0, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.0008 per cent w/v each of *betahistine mesylate IPRS* and *2- vinylpyridine* (betahistine mesylate impurity A) in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 2.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 2.0 g of *sodium dodecyl sulphate* in a mixture of 15 volumes of 10 per cent v/v solution of *sulphuric acid*, 35 volumes of 1.7 per cent w/v solution of *tetrabutylammonium hydrogen sulphate* and 650 volumes of *water*, adjusted to pH 3.3 with *sodium hydroxide solution* and 300 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks corresponding to betahistine mesylate impurity A and betahistine mesylate is not less than 3.5.

Inject reference solution (b), (c) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. The area any secondary peak corresponding to betahistine mesylate impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent). The area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

2-Propanol (5.4). Not more than 0.5 per cent.

Chlorides (2.3.12). Add 1 ml of *water* to 14 ml of solution A. The solution complies with the limit test of chlorides (35 ppm) using 2 ml of *chloride standard solution (25 ppm)*.

Sulphates (2.3.17). Dilute 6 ml of solution A in *water* to 15 ml with *water*. The solution complies with the limit test of sulphates (250 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

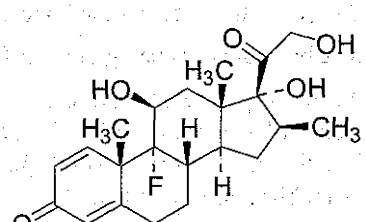
Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Assay. Dissolve 0.14 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid* and 7 volumes of *acetic anhydride* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01642 g of $C_{10}H_{20}N_2O_6S_2$.

Storage. Store protected from moisture.

Betamethasone



$C_{22}H_{29}FO_5$ Mol. Wt. 392.5

Betamethasone is 9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione.

Betamethasone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{22}H_{29}FO_5$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white to creamy-white powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betamethasone IPRS* or with the reference spectrum of betamethasone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (such as Merck silica gel 60 F254).

Mobile phase. A mixture of 85 volumes of *ether*, 10 volumes of *toluene* and 5 volumes of *1-butanol* saturated with *water*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of a mixture of 90 volumes of *chloroform* and 10 volume of *methanol*.

Reference solution (a). A 0.25 per cent w/v solution of *betamethasone IPRS* in a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Reference solution (b). A 0.125 per cent w/v solution of each of the substance under examination and *betamethasone IPRS* in the same solvent mixture.

Reference solution (c). A 0.125 per cent w/v solution of each of the substance under examination and *dexamethasone IPRS* in the same solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with *ethanolic sulphuric acid (20 per cent)*. Heat at 120° for 10 minutes or until spots are produced, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in daylight, in fluorescence in ultraviolet light at 365 nm, position and size to the principal spot in the chromatogram obtained with reference solution (a) and the chromatogram obtained with reference solution (b) shows only one spot. The test is not valid unless the chromatogram obtained with reference solution (c) shows two principal spots that are close to one another but separated.

C. Heat 0.5 ml of *chromic-sulphuric acid* in a test-tube (5 cm × about 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

D. Place 2 ml of a 0.01 per cent w/v solution in *ethanol* in a stoppered tube, add 10 ml of *phenylhydrazine solution*, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at about 450 nm (2.4.7), not more than 0.25.

Tests

Specific optical rotation (2.4.22). + 114.0° to + 122.0°, determined in a 0.5 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 240 nm, 0.37 to 0.40.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25.0 mg of the substance under examination in a mixture of equal volumes of *acetonitrile* and *methanol* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 2 mg of *betamethasone IPRS* and 2 mg of *methylprednisolone IPRS* in mobile phase A and dilute to 100.0 ml with the same mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: A. a 25 per cent v/v of *acetonitrile*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
15	100	0
40	0	100
41	100	0
46	100	0

The retention times of methylprednisolone is about 11.5 minutes and of betamethasone is about 12.5 minutes.

Inject reference solution (a). The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and betamethasone is not less than 1.5.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any

secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than one such peak has an area more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.1 g in *ethanol (95 per cent)* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 238.5 nm (2.4.7).

Calculate the content of $C_{22}H_{29}FO_5$ taking 395 as the specific absorbance at 238.5 nm.

Storage. Store protected from light.

Betamethasone Tablets

Betamethasone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

Usual strengths. 0.5 mg; 1.0 mg.

Identification

Powder a few tablets and extract with *chloroform*. Evaporate the extract to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betamethasone IPRS* or with the reference spectrum of betamethasone.

B. Place 2 ml of a 0.01 per cent w/v solution in *ethanol* in a stoppered tube, add 10 ml of *phenylhydrazine solution*, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at about 450 nm (2.4.7), not more than 0.25.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. *Chloroform*.

Test solution. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *betamethasone IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Reference solution (c). Mix equal volumes of the test solution and a 0.25 per cent w/v solution of *dexamethasone IPRS* in the solvent mixture.

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot. The chromatogram obtained with reference solution (c) shows two closely running spots.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water* and 1 ml of 0.05 per cent w/v solution of *testosterone IPRS* (internal standard) in *methanol*,

Speed and time. 50 rpm and 45 minutes.

Use one tablet in the vessel for each test.

Withdraw a suitable volume of the medium and filter.

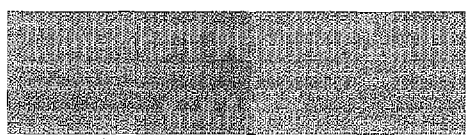
Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. Dilute a mixture of 1.0 ml each of a 0.05 per cent w/v solution of *betamethasone IPRS* in *methanol* and 1 ml of a 0.05 per cent w/v solution of *testosterone IPRS* in *methanol* to 900 ml with *water*. Make suitable changes in concentration of *betamethasone IPRS* as per test concentration.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *methanol* and 40 volumes of *water*,



- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{29}FO_5$.

Related substances. Transfer a quantity of the powdered tablets containing about 2 mg of Betamethasone to a glass-stoppered 50-ml centrifuge tube. Pipette 20 ml of *ethanol (95 per cent)* into the tube, shake for 2 minutes and allow to stand for 20 minutes with occasional shaking. Centrifuge the mixture for 5 minutes. Pipette 10 ml of the clear supernatant liquid into a glass-stoppered tube and evaporate the *ethanol* on a water-bath with the aid of a current of air to about 0.5 ml, then evaporate without heat to dryness. Pipette 1 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*, insert the stopper and mix. Centrifuge, if necessary, to remove any insoluble material. Use the solution as the test solution.

Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (such as Merck silica gel 60 F254).

Mobile phase. A mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water*.

Reference solution (a). A 0.002 per cent w/v solution of *betamethasone IPRS* in a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Reference solution (b). A 0.001 per cent w/v solution of *betamethasone IPRS* in the same solvent mixture.

Reference solution (c). A 0.1 per cent w/v solution of each of *betamethasone IPRS* and *dexamethasone IPRS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Uniformity of content. Complies with the test stated under Tablets. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Finely crush one tablet, add 20.0 ml of a 0.002 per cent w/v solution of *hydrocortisone* (internal standard) in *methanol (50 per cent)*, shake for 10 minutes and filter through a glass-fibre filter paper.

Reference solution. A solution containing 0.0025 per cent w/v of *betamethasone IPRS* and 0.002 per cent w/v of *hydrocortisone*.

Chromatographic system

- a stainless steel column 20 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 53 volumes of *water* and 47 volumes of *methanol*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 20 µl.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 2.5 mg of Betamethasone, add 20.0 ml of *methanol (50 per cent)*, shake for 10 minutes and filter through a glass-fibre paper.

Reference solution (a). A solution containing 0.0125 per cent w/v of *betamethasone IPRS* and 0.01 per cent w/v of *hydrocortisone* (internal standard) in *methanol* (50 per cent).

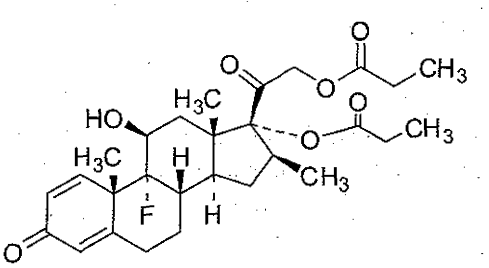
Reference solution (b). Prepare in the same manner as the test solution but use 20.0 ml of a 0.01 per cent w/v solution of *hydrocortisone* in *methanol (50 per cent)* in place of 20.0 ml of *methanol (50 per cent)*.

Use chromatographic system as described under Uniformity of content.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets.

Storage. Store protected from light.

Betamethasone Dipropionate



$C_{28}H_{37}FO_7$ Mol. Wt. 504.6

Betamethasone Dipropionate is 9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17α,21-dipropionate.

Betamethasone Dipropionate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{28}H_{37}FO_7$, calculated on the dried basis.

Category. Topical Steroid.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betamethasone dipropionate IPRS* or with the reference spectrum of betamethasone dipropionate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 10 volumes of *methanol* and 90 volumes of *dichloromethane.*

Mobile phase. Add 1.2 volumes of *water* and 8 volumes of *methanol* in a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane.*

Test solution. Dissolve 10 mg of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *betamethasone dipropionate IPRS* in the solvent mixture.

Reference solution (b). A 0.1 per cent w/v solution of *betamethasone acetate IPRS* in the solvent mixture. Dilute 5.0 ml of the solution to 10 ml with reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). Spray with *ethanolic sulphuric acid.* Heat at 120° for 10 minutes or until the spots appear, allow to cool. Examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). Reference solution (b) gives two clearly separated spots.

Tests

Specific optical rotation (2.4.22). + 63° to + 70°, determined on a 1.0 per cent w/v solution of *dioxan.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 62.5 mg of the substance under examination in 25.0 ml of the mobile phase.

Reference solution (a). Dissolve 2.5 mg of *betamethasone dipropionate RS* and 2.5 mg of *anhydrous beclomethasone dipropionate IPRS* in 50.0 ml of the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to betamethasone dipropionate and beclomethasone dipropionate is not less than 2.5.

Inject reference solution (b) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent) and not more than one such peak has an area more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The sum of all the secondary peaks is not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Dissolve 50 mg in 100.0 ml of *ethanol (95 per cent).* Dilute 2.0 ml of the solution to 50.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{28}H_{37}FO_7$ taking 305 as the specific absorbance at 240 nm.

Storage. Store protected from light.

Betamethasone Cream

Betamethasone Dipropionate Cream

Betamethasone Cream contains an amount of betamethasone dipropionate, $C_{28}H_{37}FO_7$ equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$ in a suitable cream base.

Usual strength. 0.05 per cent w/w.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 70 volumes of *chloroform* and 10 volumes of *acetone.*

Test solution. Transfer about 1.5 g of Cream to a glass-stoppered, 50-ml centrifuge tube. Add 15 ml of a *methanolic hydrochloric acid solution* prepared by mixing 1 volume of *dilute hydrochloric acid* (1 in 120) with 4 volumes of *methanol.* Shake to obtain a homogeneous mixture. Add 30 ml of *hexane,* mix for 10 minutes, and centrifuge. Using a suitable syringe, transfer the lower aqueous phase to a second centrifuge tube, add about 20 ml of *water* and mix. Extract this aqueous mixture with chloroform by shaking, centrifuging, and removing the lower, chloroform phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform to obtain a solution containing about 150 µg of betamethasone dipropionate per ml.

Reference solution. A 0.015 per cent w/v solution of *betamethasone dipropionate IPRS* in *chloroform.*

Apply to the plate 40 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Cream.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.1 per cent v/v solution of *acetic acid* in *methanol.*

Internal standard solution. A 0.045 per cent w/v solution of *beclomethasone dipropionate IPRS* in the solvent mixture.

Test solution. Weigh and transfer the cream containing 2.0 mg of of Betamethasone Dipropionate into a capped 50.0 ml centrifuge tube, add 10.0 ml of solvent mixture followed by 5.0 ml of internal standard solution and mix. Heat in a water bath maintained at 60°, shaking intermittently, until the cream melts. Remove from the bath, and shake vigorously until the specimen has solidified. Repeat the heating and shaking. Freeze in an ice-methanol bath for about 15 minutes, and centrifuge to obtain a clear supernatant liquid.

Reference solution. Add 5.0 ml of internal standard solution to 10.0 ml of a 0.02 percent w/v solution of *betamethasone dipropionate IPRS* in solvent mixture and mix.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 25 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of betamethasone, $C_{22}H_{29}FO_5$ in the cream by using the peak area ratio of the betamethasone dipropionate peak and the internal standard peak obtained from the reference solution and the test solution.

Storage. Preserve in collapsible tubes or tight containers and at a temperature of 30°, excursions permitted between 15° and 30°. Protect from freezing.

Betamethasone Lotion

Betamethasone Dipropionate Lotion

Betamethasone Lotion contains an amount of betamethasone dipropionate, $C_{28}H_{37}FO_7$ equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$ in a suitable lotion base.

Usual strength. 0.05 per cent w/w.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 70 volumes of *chloroform* and 10 volumes of *acetone.*

Test solution. Disperse a quantity of Lotion containing about 0.6 mg of Betamethasone Dipropionate with 10 ml of *0.1 M hydrochloric acid* and 4 ml of *chloroform* for 10 minutes. Centrifuge at 2000 rpm for about 5 minutes. Transfer the chloroform layer to a suitable vial.

Reference solution. A 0.015 per cent w/v solution of *betamethasone dipropionate IPRS* in *chloroform.*

Apply to the plate 40 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Lotions.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.1 per cent v/v solution of *acetic acid* in *methanol.*

Internal standard solution. A 0.09 per cent w/v solution of *beclomethasone dipropionate IPRS* in *chloroform.*

Test solution. Disperse a quantity of Lotion containing about 1.2 mg of Betamethasone Dipropionate with 10.0 ml of *0.1 M hydrochloric acid* in a capped 50-ml centrifuge tube. Add 2.0 ml of internal standard solution and 2.0 ml of *chloroform.* Cap and shake vigorously for about 2 minutes, or disperse on a vortex mixer for about 1 minute. Centrifuge at 2500 rpm for about 3 minutes. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 ml of *methanol,* and swirl to dissolve the residue.

Reference solution. A 0.06 per cent w/v solution of *betamethasone dipropionate IPRS* in *chloroform.* Transfer 5.0 ml of the solution to a suitable vial, and add 5.0 ml of internal standard solution to obtain a solution having known concentrations of about 0.3 mg of Betamethasone Dipropionate and about 0.45 mg of Beclomethasone Dipropionate per ml. To 10.0 ml of *0.1 M hydrochloric acid* in a capped 5-ml centrifuge tube add 4.0 ml of the prepared solution. Cap and shake vigorously for about 2 minutes, or disperse on a vortex mixer for about 1 minute. Centrifuge at 2500 rpm for about 3 minutes. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 ml of *methanol,* and swirl to dissolve the residue.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 25 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of betamethasone, $C_{22}H_{29}FO_5$ in the Lotion.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states (1) the concentrations of Betamethasone in the preparation; (2) that the preparation is intended for external use only; (3) that the contents should be shaken before use; the conditions under which the preparation should be stored.

Betamethasone Ointment

Betamethasone Dipropionate Ointment

Betamethasone Ointment contains an amount of betamethasone dipropionate, $C_{28}H_{37}FO_7$ equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$, in a suitable ointment base.

Usual strength. 0.05 per cent w/w.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 70 volumes of *chloroform* and 10 volumes of *acetone.*

Test solution. Shake about 1.5 g of Ointment with 15 ml of *methanolic hydrochloric acid solution* prepared by mixing 1 volume of *dilute hydrochloric acid* (1 in 120) with 4 volumes of *methanol.* Add 30 ml of *hexane,* mix for 10 minutes, and centrifuge. Using a suitable syringe, transfer the lower aqueous phase to a second centrifuge tube, add about 20 ml of *water,* and mix. Extract this aqueous mixture with *chloroform* by shaking, centrifuge and removing the lower, chloroform phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform to obtain a solution containing about 150 µg of betamethasone dipropionate per ml.

Reference solution. A 0.015 per cent w/v solution of *betamethasone dipropionate IPRS* in *chloroform.*

Apply to the plate 40 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Ointment.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.1 per cent v/v solution of *acetic acid* in *methanol.*

Internal standard solution. A 0.09 per cent w/v solution of *beclomethasone dipropionate IPRS* in *chloroform.*

Test solution. Disperse a quantity of Ointment containing about 2 mg of Betamethasone Dipropionate with 5.0 ml of internal standard solution and 10.0 ml of the solvent mixture. Heat in a water-bath at 70°, shaking intermittently until the ointment melts. Remove from the bath, and shake vigorously until the ointment has solidified. Repeat the heating and shaking operation. Freeze in an ice-methanol bath for about

15 minutes, and centrifuge at 2500 rpm for about 5 minutes. Transfer a portion of the supernatant to a suitable vial.

Reference solution. A 0.06 per cent w/v solution of *betamethasone dipropionate IPRS* in *chloroform*. Transfer 5.0 ml of the solution to a suitable vial, and add 5.0 ml of internal standard solution to obtain a solution having known concentrations of about 0.3 mg of Betamethasone Dipropionate and about 0.45 mg of Beclomethasone Dipropionate per ml. To 10.0 ml of *0.1 M hydrochloric acid* in a capped 5-ml centrifuge tube add 4.0 ml of the prepared solution. Cap, and shake vigorously for about 2 minutes, or disperse on a vortex mixer for about 1 minute. Centrifuge at 2500 rpm for about 3 minutes. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 ml of *methanol*, and swirl to dissolve the residue.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 25 µl.

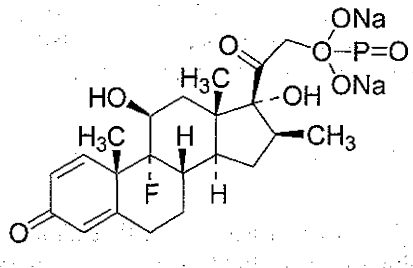
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{29}FO_5$ in the Ointment.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Betamethasone Sodium Phosphate



$C_{22}H_{28}FNa_2O_8P$ Mol. Wt. 516.4

Betamethasone Sodium Phosphate is 9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione disodium phosphate.

Betamethasone Sodium Phosphate contains not less than 96.0 per cent and not more than 103.0 per cent of $C_{22}H_{28}FNa_2O_8P$, calculated on the anhydrous basis.

Category. Adrenocortical steroid.

Description. A white or almost white powder; odourless; very hygroscopic.

Identification

A. To 2 ml of a 0.013 per cent w/v solution in *ethanol (95 per cent)* in a stoppered tube add 10 ml of *phenylhydrazine-sulphuric acid solution*, mix, warm in a water-bath at 60° for 20 minutes and cool immediately. Absorbance of the resulting solution at the maximum at about 450 nm, not more than 0.13 (2.4.7).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A freshly prepared mixture of 30 volumes of *isopropyl alcohol*, 10 volumes of *acetic acid* and 10 volumes of *water*.

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml of *water*.

Reference solution (a). A 0.25 per cent w/v solution of *betamethasone sodium phosphate IPRS* in *water*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Reference solution (c). A mixture of equal volumes of the test solution and a 0.25 per cent w/v solution of *prednisolone sodium phosphate IPRS*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes, allow to cool, and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot and the chromatogram obtained with reference solution (c) shows two closely running spots.

C. Heat 0.5 ml of *chromic-sulphuric acid* in a test-tube (5 cm x 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

D. Dissolve 2 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes; no red colour or yellowish-green fluorescence

is produced (distinction from prednisolone sodium phosphate and hydrocortisone sodium phosphate).

E. Heat gently 40 mg with 2 ml of *sulphuric acid* until white fumes are evolved, add *nitric acid* dropwise until oxidation is complete and cool. Add 2 ml of *water*, heat until white fumes are again evolved, cool, add 10 ml of *water* and neutralise to *litmus paper* with *dilute ammonia solution*. The solution gives the reactions of sodium salts and of phosphates (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 7.5 to 9.0, determined in a 0.5 per cent w/v solution.

Specific optical rotation (2.4.22). +98.0° to +104.0°, determined in a 1.0 per cent w/v solution.

Light absorption (2.4.7). Ratio of the absorbance of the solution prepared as directed under Assay at the maximum at about 241 nm to that at about 263 nm, 1.70 to 1.90.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 62.5 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.004 per cent w/v each of *betamethasone sodium phosphate IPRS* and *dexamethasone sodium phosphate IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 1.36 g of *potassium dihydrogen phosphate* and 0.6 g of *hexylamine* in 185 ml of *water* and add 65 ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The retention time of betamethasone sodium phosphate peak is about 14 minutes and of dexamethasone sodium phosphate peak is about 15.5 minutes.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to betamethasone sodium phosphate and dexamethasone sodium phosphate is not less than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and not more than one such peak has an area more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). Ignore any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Inorganic phosphate. Not more than 0.5 per cent, calculated as PO_4, determined by the following method. Weigh 25 mg, dissolve in 10 ml of *water*, add 4 ml of *dilute sulphuric acid*, 1 ml of *ammonium molybdate solution* and 2 ml of *methylaminophenol with sulphite solution* and allow to stand for 15 minutes. Add sufficient *water* to produce 25.0 ml, allow to stand for further 15 minutes and measure the absorbance of the resulting solution at the maximum at about 730 nm (2.4.7). Calculate the content of phosphate from a calibration curve prepared by treating suitable aliquots of a 0.00143 per cent w/v solution of *potassium dihydrogen phosphate* in a similar manner.

Free betamethasone and other derivatives. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. *Methanol*.

Test solution. Dissolve 1.0 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 1.0 per cent w/v solution of *betamethasone sodium phosphate IPRS* in *methanol*.

Reference solution (b). A 0.02 per cent w/v solution of *betamethasone IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air for 5 minutes and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution other than that corresponding to *betamethasone sodium phosphate IPRS* is not more intense than the spot in the chromatogram obtained with reference solution (b).

Water (2.3.43). Not more than 8.0 per cent, determined on 0.5 g.

Assay. Weigh 0.2 g and dissolve in sufficient *water* to produce 200.0 ml. Dilute 5.0 ml to 250.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7). Calculate the content of $C_{22}H_{28}FNa_2O_8P$, taking 297 as the specific absorbance at 241 nm.

Storage. Store protected from light and moisture.

Betamethasone Eye Drops

Betamethasone Eye Drops are a sterile solution of Betamethasone Sodium Phosphate in Purified Water.

Betamethasone Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone sodium phosphate, $C_{22}H_{28}FNa_2O_8P$.

Usual strength. 0.1 per cent w/v.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *butanol*, 20 volumes of *acetic anhydride* and 20 volumes of *water*.

Test solution. Dilute the eye drops suitably with *water* to get a solution containing 0.1 per cent w/v of Betamethasone Sodium Phosphate.

Reference solution (a). A 0.1 per cent w/v solution of *betamethasone sodium phosphate IPRS* in *water*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Reference solution (c). A mixture of equal volumes of reference solution (a) and 0.1 per cent w/v of *prednisolone sodium phosphate IPRS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air, heat at 110° for 10 minutes and examine under ultraviolet light at 254 nm. The chromatograms obtained with the test solution, reference solution (a) and reference solution (b) show single principal spots with similar R_f values. The chromatogram obtained with reference solution (c) shows two principal spots with almost identical R_f values.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. To a volume containing 0.2 mg of Betamethasone Sodium Phosphate, add slowly 1 ml of *sulphuric acid* and allow to stand for 2 minutes. A brownish yellow colour but no red colour or yellowish green fluorescence is produced.

Tests

pH (2.4.24). 7.0 to 8.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the eye drops if necessary to obtain a solution containing 0.1 per cent w/v of Betamethasone Sodium Phosphate.

Reference solution (a). Dilute 1 volume of the test solution to 50 volumes with *water*.

Reference solution (b). A solution containing 0.006 per cent w/v each of *betamethasone sodium phosphate IPRS* and *betamethasone IPRS*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- column temperature: 60°,
- mobile phase: a mixture of 60 volumes of *citro-phosphate buffer pH 5.0* and 40 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 241 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to betamethasone sodium phosphate and betamethasone is at least 3.5.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to betamethasone is not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a), the area of any other secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) and the sum of the areas of all the secondary peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a).

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Mix a quantity of the eye drops containing 5 mg of Betamethasone Sodium Phosphate with 10 ml of *methanol* and dilute to 25.0 ml with *water*.

Test solution (b). Mix a quantity of the eye drops containing 5 mg of Betamethasone Sodium Phosphate with 10 ml of a 0.06 per cent w/v solution of *hydrocortisone* (internal standard) in *methanol* and dilute to 25.0 ml with *water*.

Reference solution. Mix 5.0 ml of a 0.1 per cent w/v solution of *betamethasone sodium phosphate IPRS* in *water* (solution A) and 10 ml of the internal standard solution and dilute to 25.0 ml with *water*.

Chromatographic system

- a stainless steel column 20 cm x 5 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),

- mobile phase: a mixture of 55 volumes of *citrophosphate buffer pH 5.0* and 45 volumes of *methanol*,
- flow rate: 2 ml per minute.
- spectrophotometer set at 241 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution, test solution (a) and (b).

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in the eye drops.

Storage. Store protected from light.

Betamethasone Injection

Betamethasone Sodium Phosphate Injection

Betamethasone Injection is a sterile solution of Betamethasone Sodium Phosphate in Water for Injections.

Betamethasone Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

Usual strength. The equivalent of 4 mg of betamethasone per ml. (5.2 mg of Betamethasone Sodium Phosphate is approximately equivalent to 4 mg of betamethasone).

Description. A clear, colourless solution.

Identification

A. To a volume of injection containing 4 mg of betamethasone, add 1 ml of *water* and sufficient *ethanol* to produce 40 ml. To 2 ml of the solution in a stopper tube, add 10 ml of *phenyl hydrazine solution*, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at the maximum at 450 nm (2.4.7), not more than 0.1.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *1-butanol*, 20 volumes of *acetic anhydride* and 20 volumes of *water*.

Test solution. Dilute a volume of the injection with *water* to obtain a solution containing 0.2 per cent w/v of Betamethasone.

Reference solution (a). A 0.25 per cent w/v solution of *betamethasone sodium phosphate IPRS* in *water*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Reference solution (c). A mixture of equal volumes of the test solution and a 0.25 per cent w/v solution of *prednisolone sodium phosphate IPRS* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, heat at 110° for 10 minutes and examine under ultraviolet light at 254 nm. The chromatogram obtained with the test solution, reference solution (a) and reference solution (b) shows single principal spot with identical Rf values. The chromatogram obtained with reference solution (c) shows two principal spots with almost identical Rf values.

C. Evaporate a volume containing the equivalent of 2 mg betamethasone to dryness on a water-bath, dissolve the residue in 2 ml of *sulphuric acid* and allow to stand for 2 minutes, No red colour is produced.

Tests

pH (2.4.24). 8.0 to 9.0.

Appearance of solution. A 0.2 per cent w/v solution of betamethasone is not more intensely coloured than reference solution BYS4 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Dilute a volume of the injection with the mobile phase to obtain a solution containing 0.1 per cent w/v of Betamethasone.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.006 per cent w/v, each of, *betamethasone sodium phosphate IPRS* and *betamethasone IPRS* in the mobile phase

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm), (Such as Spherisorb ODS 1),
- column temperature: 60°,
- mobile phase: a mixture of 60 volumes of *citrophosphate buffer pH 5.0* and 40 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set 241 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to betamethasone sodium phosphate and betamethasone is not less than 3.5.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. The area of any peak corresponding to betamethasone is not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.6 per cent), the area of any other secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram

obtained with reference solution (a) (3.0 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent). Ignore any peak with an area less than 0.05 times of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Dilute a volume of the injection containing 8 mg of betamethasone to 50.0 ml with *methanol* (50 per cent).

Reference solution. A 0.045 per cent w/v solution of *betamethasone sodium phosphate IPRS* in *water*. Dilute 5.0 ml of the solution to 10.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm), (Such as Spherisorb ODS 1),
- column temperature: 60°,
- mobile phase: a mixture of 55 volumes of *citro-phosphate pH 5.0* and 45 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set 241nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{29}FO_5$ in the injection.

Storage. Store protected from light, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of betamethasone.

Betamethasone Sodium Phosphate Tablets

Betamethasone Sodium Phosphate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

Usual strength. The equivalent of 0.5 mg of betamethasone (0.65 mg of Betamethasone Sodium Phosphate is approximately equivalent to 0.5 mg of betamethasone).

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A freshly prepared mixture of 30 volumes of *1-butanol*, 10 volumes of *acetic anhydride* and 10 volumes of *water*.

Test solution. Dissolve a quantity of the powdered tablets containing 2 mg of betamethasone in 25 ml of *water*, add 2.5 g of *sodium chloride* and 1 ml of *hydrochloric acid*, extract with 25 ml of *chloroform* and discard the chloroform layer. Extract with 2.5 ml of *tributyl phosphate* and discard the aqueous layer.

Reference solution (a). Prepare in the same manner as the test solution but using 2.5 mg of *betamethasone sodium phosphate IPRS* instead of the substance under examination.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Reference solution (c). A mixture of equal volumes of the test solution and a solution prepared in the same manner as the test solution but using 2.5 mg of *prednisolone sodium phosphate IPRS* instead of the substance under examination.

Apply to the plate 2 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes, allow to cool, and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot and the chromatogram obtained with reference solution (c) shows two closely running spots. Secondary spots due to excipients may also be seen in the chromatograms obtained with the test solution and reference solution (b) and (c).

B. Disperse a quantity of intact tablets containing 1 mg of betamethasone in 1 ml of *sulphuric acid* and allow to stand for 5 minutes, a pale yellow colour is produced.

Tests

Disintegration (2.5.1). Not more than 5 minutes.

Uniformity of content. Complies with the test stated under Tablets. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve one tablet as completely as possible in 5 ml of *water*, add 5 ml of *methanol* and filter. Dilute with *methanol* (50 per cent v/v) to produce a solution containing 0.0032 per cent w/v of Betamethasone Sodium Phosphate.

Reference solution. A 0.0065 per cent w/v solution of *betamethasone sodium phosphate IPRS* in *water*. Dilute 5.0 ml of the solution to 10.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS),
- column temperature: 60°,
- mobile phase: a mixture of 55 volumes of *citro-phosphate buffer pH 5.0* and 45 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 241 nm,
- injection volume: 20 µl.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Disperse a quantity of the intact tablets containing 5 mg of Betamethasone in 50 ml of *water* with the aid of ultrasound for 20 minutes, dilute to 100.0 ml with *methanol*, mix and filter.

Reference solution. Dilute 5.0 ml of a 0.014 per cent w/v solution of *betamethasone sodium phosphate IPRS* in *water* to 10.0 ml with *methanol*.

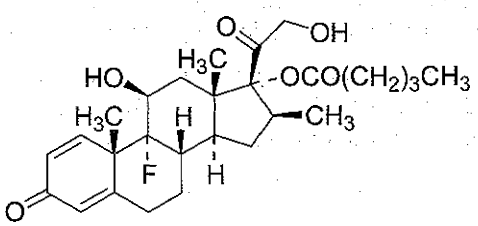
Use chromatographic system as described under Uniformity of content.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of betamethasone.

Betamethasone Valerate



$C_{27}H_{37}FO_6$ Mol. Wt. 476.6

Betamethasone Valerate is a 9α–fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione-17-valerate.

Betamethasone Valerate contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{27}H_{37}FO_6$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white to creamy-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betamethasone valerate IPRS* or with the reference spectrum of betamethasone valerate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Mobile phase. A mixture of 95 volumes of *1,2-dichloroethane*, 5 volumes of *methanol* and 0.2 volume of *water*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *betamethasone valerate IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to *betamethasone valerate IPRS* in the chromatogram obtained with the reference solution.

D. Heat 50 mg with 2 ml of *0.5 M ethanolic potassium hydroxide* in a water-bath for 5 minutes. Cool, add 2 ml of *sulphuric acid* (50 per cent v/v) and boil gently for 1 minute; the odour of ethyl valerate is perceptible.

Tests

Specific optical rotation (2.4.22). +75.0° to +82.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). Absorbance of a 0.002 per cent w/v solution in *ethanol* at the maximum at about 240 nm, 0.63 to 0.67.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 4 mg of the substance under examination add 10 ml of the mobile phase and shake well to dissolve.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 55 volumes of *acetonitrile*, 45 volumes of *water* and 0.1 volume of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the test solution. The resolution between betamethasone valerate and any impurity is not less than 1.5 and the column efficiency is not less than 9000 theoretical plates.

Inject the test solution. Calculate the content of each impurity as a percentage of the sum of all the peak responses (1.0 per cent). Not more than 2.0 per cent of total impurities is found.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 60 mg of the substance under examination, dissolve in a 0.1 percent v/v solution of *glacial acetic acid* in *methanol* and dilute to 100.0 ml with the same solvent. To 5.0 ml of the solution add 10.0 ml of reference solution (b) and mix.

Reference solution (a). Weigh a suitable quantity of *betamethasone valerate IPRS* and dissolve in a 0.1 per cent v/v solution of *glacial acetic acid* in *methanol* to obtain a solution containing a known concentration of about 0.6 mg per ml. To 5.0 ml of the solution add 10.0 ml of reference solution (b) and mix.

Reference solution (b). A 0.04 per cent w/v solution of *beclomethasone dipropionate IPRS* in a 0.1 per cent v/v solution of *glacial acetic acid* in *methanol*.

Chromatographic system
- a stainless steel column 30 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 20 volumes of *water*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

The relative retention times are about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate

Inject reference solution (a). The resolution between betamethasone valerate and beclomethasone dipropionate is not less than 4.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{37}FO_6$.

Storage. Store protected from light.

Betamethasone Valerate Cream

Betamethasone Valerate Cream contains Betamethasone Valerate in a suitable cream base.

Betamethasone Valerate Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

Ususal strengths. 0.1 per cent; 0.12 per cent.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform*.

Test solution. Disperse a quantity of the cream containing 0.5 mg of Betamethasone in 20 ml of *methanol (80 per cent)* by heating on a water-bath until the methanol begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *chloroform*, shake vigorously, allow the layers to separate and evaporate the chloroform layer to dryness in a current of nitrogen with gentle heating. Dissolve the residue in 1 ml of *chloroform*.

Reference solution (a). A 0.03 per cent w/v solution of *betamethasone valerate IPRS* in *chloroform*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air until the solvent has evaporated, heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Disperse a quantity of the cream containing 2 mg of betamethasone with 100 ml of hot *hexane* for 2 minutes, cool, extract the mixture with 20 ml of *ethanol (75 per cent)*

and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (75 per cent)*. Repeat the extraction of the hexane mixture with two 10-ml quantities of *ethanol (75 per cent)*, filtering each extract in turn through the absorbent cotton. Combine the filtrates, add 5 ml of a 0.072 per cent w/v solution of *beclometasone dipropionate IPRS* (internal standard) and dilute to 50.0 ml with *ethanol (75 per cent)*.

Test solution (b). Disperse a quantity of the cream containing 2 mg of betamethasone with 100 ml of hot *hexane* for 2 minutes, cool, extract the mixture with 20 ml of *ethanol (95 per cent)* and filter the lower, ethanolic layer through absorbent cotton previously washed with *ethanol (75 per cent)*. Repeat the extraction of the hexane mixture with two 10-ml quantities of *ethanol (75 per cent)*, filtering each extract in turn through the absorbent cotton and dilute the combined filtrates to 50.0 ml with *ethanol (75 per cent)*.

Reference solution. Mix 10 ml of a solution containing 0.024 per cent w/v of *betamethasone valerate IPRS* and 0.0012 per cent w/v of *betamethasone 21-valerate IPRS* in *ethanol (80 per cent)* with 5 ml of a 0.072 per cent w/v solution of *beclometasone dipropionate IPRS* (internal standard) in *ethanol (80 per cent)* and dilute to 50.0 ml with the same solvent.

Chromatographic system

- a stainless steel column 10 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 60°,
- mobile phase: a mixture of 42 volumes of *ethanol* and 58 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to betamethasone valerate (retention time: about 5 minutes) and betamethasone 21-valerate (retention time: about 7 minutes) is not less than 1.0.

Inject the reference solution and test solution (b).

Calculate the content of $C_{22}H_{29}FO_5$ in the cream.

Storage. Store protected from light.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of betamethasone.

Betamethasone Valerate Ointment

Betamethasone Valerate Ointment contains Betamethasone Valerate in a suitable ointment base.

Betamethasone Valerate Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

Usual strengths. The equivalent of 0.025 per cent w/w and 0.1 per cent w/w of betamethasone (120 mg of Betamethasone Valerate is approximately equivalent to 100 mg of betamethasone).

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 20 volumes of *chloroform*, 2 volumes of *acetone* and 1 volume of *ethanol*.

Test solution. Heat a quantity of the ointment containing 1 mg of betamethasone with 10 ml of *methanol* on a water-bath until it boils, shake vigorously, cool in ice for 30 minutes, filter, evaporate the filtrate to dryness in a current of nitrogen with gentle heating and dissolve the residue in 0.5 ml of *chloroform*.

Reference solution. A 0.24 per cent w/v solution of *betamethasone valerate IPRS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and spray while hot with *alkaline tetrazolium blue solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to *betamethasone valerate IPRS* in the chromatogram obtained with the reference solution.

Tests

Microbial contamination (2.2.9). 1.0 g is free from *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Other tests. Comply with the tests stated under Ointments.

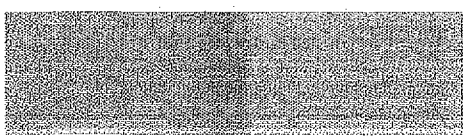
Assay. Determine by liquid chromatography (2.4.14).

Test solution. Heat a quantity of the weighed ointment containing 2.5 mg of betamethasone with 10.0 ml of 0.04 per cent w/v solution of *beclomethasone dipropionate IPRS* (internal standard) in *methanol* containing 0.1 per cent v/v of *glacial acetic acid* and 5.0 ml of *methanol* containing 0.1 per cent v/v of *glacial acetic acid* on a water-bath until it boils, shake vigorously, cool in ice for 30 minutes, centrifuge and decant the supernatant solution into a stoppered flask.

Reference solution. Mix 5 ml of a 0.06 per cent w/v solution of *betamethasone valerate IPRS* in *methanol* containing 0.1 per cent v/v of *glacial acetic acid* and 10.0 ml of a 0.04 per cent w/v solution of *beclomethasone dipropionate IPRS* in *methanol* containing 0.1 per cent v/v of *glacial acetic acid*.

Chromatographic system

- a stainless steel column 30 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),



- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

The relative retention time with reference to betamethasone valerate for beclomethasone dipropionate is about 1.7.

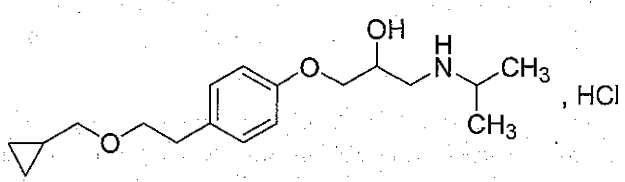
Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{29}FO_5$ in the ointment.

Storage. Store protected from light. Avoid exposure to excessive heat.

Labelling. The label states the strength in terms of the equivalent amount of betamethasone.

Betaxolol Hydrochloride



$C_{18}H_{29}NO_3$, HCl Mol Wt. 343.9

Betaxolol Hydrochloride is (*RS*)-1-[4-[2-(Cyclopropyl methoxy)ethyl]phenoxy]-3-[(1-methylethyl)amino]propan-2-ol hydrochloride.

Betaxolol Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of the $C_{18}H_{29}NO_3$, HCl, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *betaxolol hydrochloride IPRS* or with the reference spectrum of betaxolol hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *octadecylsilane silica gel F254*.

Mobile phase. A mixture of 0.5 volume of *perchloric acid*, 50 volumes *methanol* and 50 volumes of *water*.

Test solution. Dissolve 10 mg of the substance under examination in 1 ml of *methanol*.

Reference solution (a). A 1.0 per cent w/v solution of *betaxolol hydrochloride IPRS* in *methanol*.

Reference solution (b). A 1.0 per cent w/v solution of *oxprenolol hydrochloride IPRS* in reference solution (a).

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine under ultraviolet light at 254 nm and spray with 5 per cent w/v solution of *vanillin* in a mixture of 5 volumes of *sulphuric acid*, 10 volumes of *glacial acetic acid* and 85 volumes of *methanol*, heat at 105° and examine in day light. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. Dissolve 0.2 g in 20 ml of *carbon dioxide-free water*, add 0.2 ml of *methyl red solution* and 0.2 ml of *0.01 M hydrochloric acid*. The solution is red. Add 0.4 ml of *0.01 M sodium hydroxide*. The solution is yellow.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Prepare the reference solutions immediately before use.

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 5.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.04 per cent w/v of the substance under examination and 0.02 per cent w/v of *betaxolol impurity A IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 175 volumes of *acetonitrile*, 175 volumes of *methanol* and 650 volumes of 0.34 per cent w/v solution of *potassium dihydrogen phosphate* in *water*, previously adjusted to pH 3.0 with *ortho-phosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 273 nm,
- injection volume: 20 µl.

Name	Relative retention time
Betaxolol impurity B[1]	0.3
Betaxolol impurity A[2]	0.8
Betaxolol (Retention time: about 8 minutes)	1.0
Betaxolol impurity D[3]	1.5
Betaxolol impurity E[4]	2.2
Betaxolol impurity C[5]	4.1

[1](2*RS*)-1-[4-(2-hydroxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,

[2] (2*RS*)-1-(4-ethylphenoxy)-3-[(1-methylethyl)amino]propan-2-ol,

[3] 4-[2-(cyclopropylmethoxy)ethyl]phenol,

[4](2*RS*)-1-[4-(2-butoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,

[5](2*RS*)-2-[[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]methyl]oxirane.

Inject reference solution (a). The test is not valid unless the resolution between the peaks corresponding to betaxolol hydrochloride impurity A and betaxolol is not less than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram for 4.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area any peak corresponding to betaxolol impurities A, B, C, D and E is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.3 g in 10 ml of *0.01 M hydrochloric acid* and add 50 ml of *ethanol (95 per cent)*. Titrate with *0.1 M sodium hydroxide*, determining the end- point potentiometrically (2.4.25). Carry out a blank titration. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03439 g of $C_{18}H_{30}ClNO_3$.

Storage. Store protected from light.

Betaxolol Eye Drops

Betaxolol Eye Drops are a sterile solution of Betaxolol Hydrochloride in Purified Water.

Betaxolol Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betaxolol, $C_{18}H_{29}NO_3$.

Usual strengths. 0.25 per cent w/v; 0.5 per cent w/v.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 30 volumes of a solution prepared by diluting 1 volume of *13.5 M ammonia* to 50 volumes with *propan-2-ol* immediately before use and 70 volumes of *chloroform*.

Test solution. Dilute the eye drops with *water* to obtain a solution containing 0.1 per cent w/v of betaxolol. Shake 1 ml of the solution with 4 ml of *water*, 0.1 ml of *13.5M ammonia* and 2 ml of *chloroform*, centrifuge and use the chloroform layer.

Reference solution (a). A 0.1 per cent w/v solution of *betaxolol hydrochloride IPRS* in *water*. Shake 1 ml of solution with 4 ml of *water*, 0.1 ml of *13.5 M ammonia* and 2 ml of *chloroform*, centrifuge and use the chloroform layer.

Reference solution (b). A mixture of equal volumes of test solution and reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with a solution prepared by dissolving 5 g of *iodine* and 10 g of *potassium iodide* in sufficient *water* to produce 100 ml and mixing 20 ml of the resulting solution with 30 ml of *water* and 50 ml of *2 M acetic acid*. Examine the plate immediately; spots due to betaxolol is brown colour. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows a single, compact spot

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 6.0 to 7.8.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the eye drops to obtain a solution containing the equivalent of 0.02 per cent w/v of betaxolol in the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: dissolve 3 g of *sodium dodecyl sulphate* in 450 ml of the solution containing 45 volumes of a buffer solution prepared by diluting 5 ml of *orthophosphoric acid* to 990 ml of *water*, adjusted the pH to 3.0 with *2 M ammonia* and diluted to 1000 ml with *water* and 55 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 8000 theoretical plates and the tailing factor is not more than 2.5.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent), the area of not more than one secondary peak is more than 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent).

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the eye drops to obtain a solution containing 0.01 per cent w/v of betaxolol in the mobile phase.

Reference solution (a). A 0.012 per cent w/v solution of *betaxolol hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.012 per cent w/v of *betaxolol hydrochloride IPRS* and 0.006 per cent w/v solution of *pilocarpine nitrate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica,
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water* containing 0.71 per cent w/v of *anhydrous disodium hydrogen orthophosphate* and 0.91 per cent w/v solution of *dimethylamine hydrochloride*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

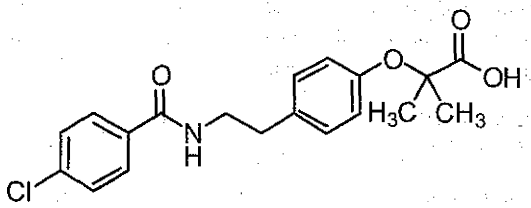
Injection reference solution (b). The test is not valid unless the resolution between the peaks coressponding to betaxolol and pilocarpine is not less than 1.5.

Calculate the content of $C_{18}H_{29}NO_3$ in the eye drops.

Storage. Store protected from light.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of betaxolol.

Bezafibrate



$C_{19}H_{20}ClNO_4$ Mol. Wt. 361.8

Bezafibrate is 2-[4-[2-(4-Chlorobenzamido)ethyl]phenoxy]-2-methylpropanoic acid.

Bezafibrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{20}ClNO_4$, calcuated on the dried basis.

Category. Hypolipidaemic.

Description. A white or almost white, crystalline powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bezafibrate IPRS* or with the reference spectrum of bezafibrate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2.7 volumes of *glacial acetic acid*, 30 volumes of *methyl ethyl ketone* and 60 volumes of *xylene*.

Test solution. Dissolve 10 mg of the substance under examination in *methanol* and dilute to 5 ml with *methanol*.

Reference solution. A 0.2 per cent w/v solution of *bezafibrate IPRS* in *methanol*.

Apply to the plate 5 µl of each solution. After development dry the plate in air and heat at 120° for 15 minutes, allow to cool and examine under ultraviolet light at 254 nm. The principal

spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *dimethylformamide* (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution BYS4 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 10.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c). To 1.0 ml of the test solution, add 1 ml of *0.1 M hydrochloric acid* and evaporate to dryness on a hot plate. Dissolve the residue in 20.0 ml of the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *methanol* and 40 volumes of a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 2.3 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume: 20 µl,

Name	Relative retention time
Bezafibrate impurity A[1]	0.5
Bezafibrate impurity B[2]	0.6
Bezafibrate (Retention time: about 6 minutes)	1.0
Bezafibrate impurity C[3]	1.5
Bezafibrate impurity D[4]	2.3
Bezafibrate impurity E[5]	6.2

[1]chlorobenzoyltyramine,

[2]4-chlorobenzoic acid,

[3]methyl 2-[4-[2-[(4-chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoate,

[4]ethyl 2-[4-[2-[(4-chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoate,

[5]butyl 2-[4-[2-[(4-chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoate.

Inject reference solution (b) and (c). The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with reference solution (c) is not less than 5.0. The signal to noise ratio for the principal peak in the chromatogram obtained with the reference solution (b) is not less than 5.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak corresponding to each of the bezafibrate impurities A, B, C, D and E is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).The sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides (2.3.12). Boil 0.83 g with 30 ml of *water* for 5 minutes, cool and filter. The filtrate complies with the limit test for chlorides (300 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g drying in an oven at 105°.

Assay. Dissolve 0.3 g in a 50 ml mixture of 25 volumes of *water* and 75 volumes of *ethanol (95 per cent)*. Titrate with *0.1 M sodium hydroxide* until a pink colour is obtained, using 0.1 ml of *phenolphthalein solution* as an indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03618 g of $C_{19}H_{20}ClNO_4$.

Bezafibrate Tablets

Bezafibrate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bezafibrate, $C_{19}H_{20}ClNO_4$.

Usual strengths. 200 mg; 400 mg.

Identification

Disperse a quantity of the powdered tablets containing 0.2 g of Bezafibrate with two 10 ml quantities of *acetone* for 10 minutes, combine and filter the extracts and evaporate the filtrate to dryness. On the residue, determine by infrared

absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *bezafibrate IPRS* or with the reference spectrum of bezafibrate.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of buffer solution pH 6.5 prepared by dissolving 0.608 g of *sodium hydroxide* and 6.805 g of *potassium dihydrogen orthophosphate* in sufficient *water* to produce 1000 ml and adjusted to pH 6.5 with *sodium hydroxide solution* or *orthophosphoric acid*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium, filter and dilute with the dissolution medium, if necessary. Measure the absorbance of the solution, at the maximum at about 229 nm (2.4.7). Calculate the content of $C_{19}H_{20}ClNO_4$ in the medium from the absorbance of a 0.0011 per cent w/v solution of *bezafibrate IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{19}H_{20}ClNO_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.1 g of Bezafibrate in 15 ml of *methanol* with the aid of ultrasound for 2 minutes, shake for a further 10 minutes, and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.0002 per cent w/v each of *bezafibrate IPRS* and *chlorobenzoyltyramine IPRS* prepared by dissolving in minimum quantity of *methanol* and dilute with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 3.9 volumes of 40 per cent w/v of *tetrabutylammonium hydroxide*, 400 volumes of *acetonitrile* and 600 volumes of *water* and adjusted to pH 4.0 with 10 per cent v/v *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume: 20 µl.

The retention time of bezafibrate is about 5 minutes.

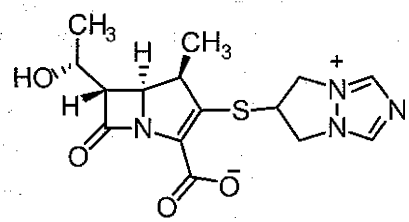
Inject reference solution (c). The test is not valid unless, the resolution between the peaks corresponding to bezafibrate and chlorobenzoyltyramine is not less than 7.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent). Ignore any peak with an area less than that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 100 mg of Bezafibrate in 70 ml of *methanol* with the aid of ultrasound for 2 minutes, shake for a further 10 minutes and dilute 100 ml with *methanol* and filter. Dilute 1.0 ml of the solution to 100.0 ml with *methanol* and measure the absorbance of the solution at the maximum at about 229 nm (2.4.7). Calculate the content of $C_{19}H_{20}ClNO_4$ from the absorbance of 0.001 per cent w/v solution of *bezafibrate IPRS* in *methanol*.

Biapenem



$C_{15}H_{18}N_4O_4S$ Mol Wt. 350.4

Biapenem is (4*R*,5*S*,6*S*)-3-(6,7-dihydro-5*H*-pyrazolo[1,2-a][1,2,4] triazol-8-ium-6-yl sulfanyl-6-(1-hydroxyethyl)-4, 7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2 carboxylate.

Biapenem contains not less than 95.0 per cent and not more than 101.0 per cent of of $C_{15}H_{18}N_4O_4S$, calculated on the anhydrous basis.

Category. Antibiotic.

Description. A white to pale yellow powder.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.0 to 6.0, determined on 1.0 per cent w/v solution in *water*.

Specific optical rotation (2.4.22). −35° to −25°, determined on 1.0 per cent w/v solution in *phosphate buffer pH 7.0*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 75 mg of the substance under examination in *water* and dilute to 25.0 ml with *water*.

Reference solution (a). A 0.003 per cent w/v solution of *biapenem IPRS* in *water*.

Reference solution (b). Dissolve 30 mg of *biapenem IPRS* in 1.0 ml of *sodium hydroxide solution* and dilute to 10.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.284 per cent w/v solution of *disodium hydrogen orthophosphate* in *water*, adjusted to pH 7.0 with *orthophosphoric acid* ,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	97	3
7	97	3
25	80	20
30	70	30
34	70	30
35	97	3
45	97	3

The relative retention time with reference to biapenem for base degraded impurity is about 0.38.

Inject reference solution (b). The test is not valid unless the resolution between base degraded impurity and biapenem is not less than 6.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (a) (1.0 per cent) and the sum of areas of all the peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (a) (3.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Bacterial endotoxins (2.2.3). Not more than 1.17 Endotoxin units per mg of biapenem.

Sterility (2.2.11). Complies with the test for sterility.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *biapenem IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 98 volumes of buffer solution prepared by dissolving 1.54 g *ammonium acetate* in 1000 ml of *water* and 2 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 20 µl.

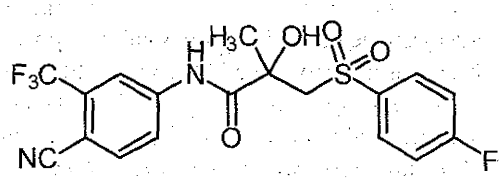
Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{18}N_4O_4S$.

Storage. Store protected from moisture.

Bicalutamide



$C_{18}H_{14}F_4N_2O_4S$ Mol. Wt. 430.4

Bicalutamide is (*RS*)-*N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide.

Bicalutamide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{14}F_4N_2O_4S$, calculated on the dried basis.

Category. Antineoplastic.

Description. A white to pale yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bicalutamide IPRS* or with the reference spectrum of bicalutamide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). −0.5 to +0.5, determined on 1.0 per cent w/v solution in *ethyl acetate* at 25°.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 1 volume of mobile phase A and 2 volumes of mobile phase B.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.0001 per cent w/v solution of *bicalutamide IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0005 per cent w/v of *bicalutamide related compound A IPRS* and 0.005 per cent w/v of *bicalutamide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a 0.01 per cent v/v of *trifluoroacetic acid* in *water*,
  B. a 0.01 per cent v/v of *trifluoroacetic acid* in *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	67	33
16.5	67	33
26.5	40	60
32.5	5	95
32.6	67	33
35	67	33

Name	Relative retention time	Correction factor
Bicalutamide aminobenzonitrile[1]	0.30	0.71
Bicalutamide impurity A isomer A[2]	0.64	—
Bicalutamide impurity A isomer B[2]	0.67	—
Desfluoro bicalutamide[3]	0.83	0.91
2-Fluoro bicalutamide[4]	0.94	—
Bicalutamide	1.00	—
Deoxybicalutamide[5]	1.33	—
Bicalutamide sulphide[6]	1.56	—

[1]4-Amino-2-(trifluoromethyl)benzonitrile,

[2]*N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfinyl]-2-hydroxy-2-methylpropanamide,

[3] *N*-[4-Cyano-3-(trifluoromethyl)phenyl]-2-hydroxy-2-methyl-3-(phenylsulfonyl)propanamide,

[4]*N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(2-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamide,.

[5]*N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylsulfonyl)-2-methylpropanamide,

[6]*N*-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylthio)-2-hydroxy-2-methylpropanamide.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to bicalutamide related compound A isomer A and isomer B is not less than 0.8 and between the peaks due to bicalutamide related compound A isomer B and bicalutamide is not less than 8.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to bicalutamide aminobenzonitrile, bicalutamide related compound A isomer A, isomer B and bicalutamide sulphide, each of, is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any peak corresponding to desfluoro bicalutamide, 2-fluoro bicalutamideand deoxybicalutamide, each of, is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphates (2.3.17). 0.3 g complies with the test for sulphates (500 ppm).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *bicalutamide IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{14}F_4N_2O_4S$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Bicalutamide Tablets

Bicalutamide Tablets contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bicalutamide $C_{18}H_{14}F_4N_2O_4S$.

Usual strengths. 50 mg; 150 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 1000 ml of 1.0 per cent w/v *sodium lauryl sulphate* in *water*,
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 272 nm (2.4.7). Calculate the content of $C_{18}H_{14}F_4N_2O_4S$ in the medium from the absorbance obtained from a solution of known concentration of *bicalutamide IPRS*.

Q. Not less than 70 per cent of the stated amount of $C_{18}H_{14}F_4N_2O_4S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 50 mg of Bicalutamide with 30 ml of mobile phase with the aid of ultrasound for about 10 minutes and dilute to 50.0 ml with mobile phase.

Reference solution. A 0.001 per cent w/v solution of *bicalutamide IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 7.1 g of *anhydrous disodium hydrogen orthophosphate* and 1.0 g of *hexane sodium sulphonate* in 1000 ml *water*, adjusted the pH to 7.3 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Water (2.3.43). Not more than 6.0 per cent, determined on 0.5 g.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Bicalutamide with 30 ml of *methanol* with the aid of ultrasound for 10 minutes and dilute to 50.0 ml with *methanol*. Further dilute 5.0 ml of the solution to 50.0 ml with *methanol*.

Reference solution. A 0.01 per cent w/v solution of *bicalutamide IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 7.1 g of *anhydrous disodium hydrogen orthophosphate* and 1.0 g of *hexane sodium*

sulphonate in 1000 ml *water*, adjusted to pH 7.3 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

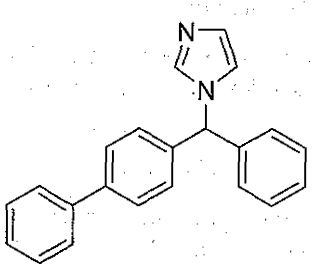
Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{14}F_4N_2O_4S$ in the tablets.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the amount of bicalutamide.

Bifonazole



$C_{22}H_{18}N_2$ Mol. Wt. 310.4

Bifonazole is 1-[(*RS*)-(biphenyl-4-yl)phenylmethyl]-1*H*-imidazole

Bifonazole contains not less than 98.0 per cent and not more than 100.5 per cent of $C_{22}H_{18}N_2$, calculated on the dried basis.

Category. Antifungal.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bifonazole IPRS* or with the reference spectrum of bifonazole.

Tests

Optical rotation (2.4.22). – 0.1° to + 0.1°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determined by liquid chromatography (2.4.14).

Buffer solution pH 3.2. Mix 2.0 ml of *orthophosphoric acid* with *water* and dilute to 1000.0 ml with the same solvent. Adjusted to pH 3.2 with *triethylamine*.

Test solution. Dissolve 50 mg of the substance under examination in 25 ml of *acetonitrile* and dilute to 50.0 ml with buffer solution pH 3.2.

Reference solution. Dilute 0.25 ml of the test solution to 50.0 ml with buffer solution pH 3.2.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 20 volumes of *acetonitrile* and 80 volumes of *buffer solution pH 3.2*,
  B. a mixture of 20 volumes of *buffer solution pH 3.2* and 80 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
8	60	40
12	10	90
30	10	90
32	60	40

Inject the reference solution and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent). Sum of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore the peaks having area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.25 g in 80 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03104 g of $C_{22}H_{18}N_2$.

Bifonazole Cream

Bifonazole Cream contains Bifonazole in a suitable cream base.

Bifonazole Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bifonazole, $C_{22}H_{18}N_2$.

Usual strength. 1.0 per cent w/w.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the cream containing about 10 mg of Bifonazole with 40 ml of *methanol* in a 50-ml volumetric flask for 30 minutes and heat on a water-bath until the sample dissolves, allow to cool and dilute to volume with *methanol*. Freeze out the fatty phase under swirling 2 minutes in an ice-bath and filter through a membrane filter.

Reference solution. A 0.02 per cent w/v solution of *bifonazole IPRS* in *methanol.*

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as LiChrospher 60 RP Select – B),
- column temperature: 40°,
- mobile phase: a mixture of 47 volumes of *acetonitrile*, 53 volumes of *0.02 M orthophosphoric acid*, adjusted to pH 5.0 with *ammonia solution*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 258 nm,
- injection volume: 10 µl.

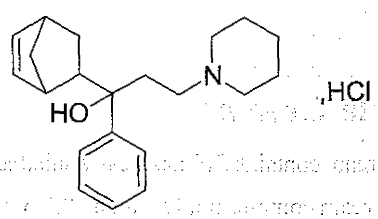
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{18}N_2$ in the cream.

Storage. Store at a temperature not exceeding 30°.

Biperiden Hydrochloride



$C_{21}H_{29}NO,HCl$ Mol. wt. 347.9

Biperiden Hydrochloride is (*RS*)-1-[(*IPRS*,2*RS*,4*RS*)-bicyclo-[2.2.1] hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride.

Biperiden Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{29}NO,HCl$, calculated on the dried basis.

Category. Anticholinergic.

Description. A white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *biperiden hydrochloride IPRS* or with the reference spectrum of biperiden hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 100 volumes of *toluene*, 5 volumes of *diethylamine* and 5 volumes of *methanol.*

Test solution. Dissolve 0.5 g of the substance under examination in 100.0 ml of *methanol.*

Reference solution (a). A 0.5 per cent w/v solution of *biperiden hydrochloride IPRS* in *methanol.*

Reference solution (b). Dissolve 5 mg of (*SR*)-1-[(1*RS*, 2*RS*, 4*RS*)-bicyclo [2.2.1] hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl]-propan-1-ol (*endo* form) in reference solution (a) and dilute to 2 ml with the same solution.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). Spray with *dilute iodobismuthate solution* and examine under daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. To about 20 mg add 5 ml of *phosphoric acid*; a green colour develops.

D. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 0.2 per cent solution in *carbon dioxide-free water* is not more opalescent than opalescence standard OS2 (2.4.1), and is colourless (2.4.1).

pH (2.4.24). 5.0 to 6.5, determined in a 0.2 per cent w/v solution.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 1.0 g of the substance under examination in 100.0 ml of *methanol*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100 ml with *methanol* and mix. Dilute 10 ml of the resulting solution to 100 ml with *methanol*.

Reference solution (b). To 1.0 ml of the test solution add 10 ml of *methanol* and 10 mg of (*SR*)-1-[(1*RS*, 2*RS*, 4*RS*)-bicyclo [2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl) propan-1-ol (*endo* form) and sufficient *methanol* to produce 100 ml.

Chromatographic system
- a fused-silica capillary column, 50 m x 0.25 mm coated with poly (vinyl-phenylmethyl siloxane with thickness of 0.25 µm,
- flame ionisation detector,
- temperature:
- column. 200° for 5 minutes, then raised at the rate of 2° per minute to 270°,
- inlet port at 250° and detector at 300°,
- flow rate: 0.4 ml per minute using nitrogen as the carrier gas and a split ratio of 1:250.

Inject 2 µl of each solution. The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the first peak due to biperiden and the second peak due to (*SR*)-1-[(1*RS*, 2*RS*,4*RS*)]-bicyclo [2.2.1] hept-5-en-2-yl]-phenyl-3- (piperidin-1-yl)propane-1-ol (*endo* form) is at least 2.5; the principal peak in the chromatogram obtained with reference solution (a) has a signal-to-noise ratio of at least 6. For peaks with a retention time of 0.95 to 1.05 relative to biperiden, the area of any peak, other than the principal peak, is not more than 0.5 per cent of the area of the principal peak and the sum of the areas of any such peaks is not more than 1.0 per cent of the area of the principal peak. For peaks with relative retention times outside the above-mentioned range, the area of any peak is not more than 0.1 per cent of the area of the principal peak and the sum of the areas of such peaks is not more than 0.5 per cent of the area of the principal peak. Ignore any peak with an area less than 0.05 per cent of the area of the principal peak in the chromatogram obtained with the test solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh 0.5 g, dissolve in 80 ml of *anhydrous glacial acetic acid*, warming slightly, if necessary to effect solution and cool. Add 10 ml of *mercuric acetate solution* and titrate with *0.1 M perchloric acid*, using 0.1ml of *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03479 g of $C_{21}H_{29}NO$, HCl.

Storage. Store protected from light.

Biperiden Tablets

Biperiden Hydrochloride Tablets

Biperiden Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of biperiden hydrochloride, $C_{21}H_{29}NO$, HCl.

Usual strength. 2 mg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution*.

Test solution. Disperse a quantity of the powdered tablets containing about 10 mg of Biperiden Hydrochloride with 5 ml of *water* and disperse the powder with the aid of ultra sound for a few minutes. Add 5 ml of *methanol* and mix again for 15 minutes. Filter the solution into a separator, add 2 ml of *1 M sodium hydroxide* and 10 ml of *chloroform* and shake for 3 minutes. Filter the chloroform layer into a stoppered flask and use the filtrate.

Reference solution. Prepare in a similar manner using 10 mg of *biperiden hydrochloride IPRS* in place of the substance under examination.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose it to iodine vapours till spots appear. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw 75 ml of the solution and filter through a membrane filter disc with an average pore diameter not greater than

1.0 µm, rejecting the first few ml of the filtrate. Transfer 50.0 ml of the clear filtrate into a suitable container, adjusted to pH 5.3 with *0.1 M sodium hydroxide*. Transfer the solution to a 100-ml volumetric flask and dilute with *water* to volume and mix.

Prepare a reference solution by weighing 80 mg of *biperiden hydrochloride IPRS* in sufficient *methanol* to produce 100.0 ml. Dilute 5.0 ml of the solution to 500.0 ml with *0.1 M hydrochloric acid* and mix. Transfer 25.0 ml of the resulting solution into a suitable container and adjusted to pH 5.3 with *0.1 M sodium hydroxide* and dilute to 100.0 ml with *water* (2 µg per ml).

Prepare a blank solution by treating 50 ml of *water* in place of the clear filtrate in the same manner as described for the test solution beginning at the words "adjusted to pH 5.3.....

Transfer 20.0 ml of the solutions into individual separators, each containing 10.0 ml of *phosphate-buffered bromocresol purple solution*. Add 40.0 ml of *chloroform* to each and shake for 10 minutes. After the layers have separated, filter each chloroform extract through a filter paper into separate, glass-stoppered flasks, discarding the first 10 ml of each filtrate.

Measure the absorbances of the solutions at the maximum at about 408 nm (2.4.7) against the blank solution. Calculate the content of $C_{21}H_{29}NO$,HCl in the medium from the absorbance obtained from the reference solution.

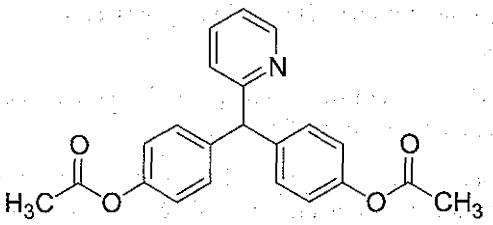
Q. Not less than 75 per cent of the stated amount of $C_{21}H_{29}NO$, HCl.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 2 mg of Biperiden Hydrochloride and transfer to a 50-ml volumetric flask, add 12.5 ml of *water* and heat on a steam-bath for 15 minutes. Cool, dilute with *methanol* to volume and mix. Transfer 5.0 ml of the resulting solution to a separator, add 10.0 ml of *phosphate-buffered bromocresol purple solution*, extract with two quantities, each of 20 ml, of *chloroform* and allow to separate. Filter the chloroform extracts into a 50-ml volumetric flask through filter paper and make to volume. Measure the absorbance of the resulting solution at the maximum at about 408 nm (2.4.7), using a reagent blank of a mixture of 3 volumes of *methanol* and 1 volume of *water* and preparing the solution in a similar manner as that of the test solution omitting the substance under examination. Calculate the content of $C_{21}H_{29}NO$, HCl from the absorbance obtained by repeating the operation using a solution prepared by adding 5.0 ml of a 0.08 per cent w/v solution of *biperiden hydrochloride IPRS* in *methanol* to 25 ml of *water*, diluting to 100.0 ml with *methanol* and treating in the same manner as the test solution.

Storage. Store protected from light.

Bisacodyl



$C_{22}H_{19}NO_4$ Mol. Wt. 361.4

Bisacodyl is bis(4-acetoxyphenyl)-2-pyridylmethane.

Bisacodyl contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{22}H_{19}NO_4$, calculated on the dried basis.

Category. Laxative.

Description. A white or almost white, crystalline powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bisacodyl IPRS* or with the reference spectrum of bisacodyl.

B. When examined in the range 230 nm to 360 nm, a 0.001 per cent w/v solution in *0.1 M potassium hydroxide* in *methanol* shows an absorption maximum only at about 248 nm, about 0.65 (2.4.7).

Tests

Acidity or alkalinity. Shake 1.0 g with 20 ml of *carbon dioxide-free water*, boil, cool and filter. Add 0.2 ml of *0.01 M sodium hydroxide* and 0.1 ml of *methyl red solution*. The resulting solution is yellow and not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 4 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 66 volumes of *water*.

Test solution. Dissolve 50 mg of substance under examination in 25 ml of *acetonitrile* and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dissolve 2 mg of *bisacodyl for system suitability IPRS* (containing bisacodyl impurity A, B, C, D and E) in 1.0 ml of *acetonitrile* and dilute to 2.0 ml with the solvent mixture.

Reference solution (c). Dissolve 5 mg of *bisacodyl for peak identification IPRS* (containing bisacodyl impurity F) in 2.5 ml of *acetonitrile* and dilute to 5.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of 0.16 per cent w/v solution of *ammonium formate*, adjusted to pH 5.0 with *anhydrous formic acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject reference solution (a). The relative retention time with reference to bisacodyl for 4,4'- (pyridine-2-ylmethylene) diphenol (bisacodyl impurity A) is about 0.2, for 2-((*RS*)-(4-hydroxyphenyl)(pyridine-2-yl)methyl)phenol (bisacodyl impurity B) is about 0.4, for 4-((*RS*)-(4-hydroxyphenyl) (pyridine-2-yl)methyl)phenyl acetate (bisacodyl impurity C) is about 0.45, for bisacodyl impurity D is about 0.8, for 2-((*RS*)-(4-acetyloxy)phenyl)(pyridine-2-yl)methyl)phenyl acetate (bisacodyl impurity E) is about 0.9, and for bisacodyl impurity F is about 2.6.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio between the peaks due to bisacodyl impurity E and bisacodyl is not less than 1.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peaks corresponding to bisacodyl impurity A, multiplied by correction factor of 0.7 and impurity B, each of, is not more than the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of secondary peak corresponding to bisacodyl impurity C and E is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of secondary peak corresponding to bisacodyl impurity D is not more than twice the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of secondary peak corresponding to bisacodyl impurity F is not more than 3 times the area of corresponding peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and sum of areas of all secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Weigh 0.3 g and dissolve in 50 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03614 g of $C_{22}H_{19}NO_4$.

Storage. Store protected from light.

Bisacodyl Suppositories

Bisacodyl Suppositories contain Bisacodyl in a suitable suppository base.

Bisacodyl Suppositories contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bisacodyl, $C_{22}H_{19}NO_4$.

Usual strengths. 5 mg; 10 mg.

Identification

A. Dissolve a quantity of the suppositories containing 0.15 g of Bisacodyl as completely as possible in 150 ml of *light petroleum (40° to 60°)*, filter, wash the residue with *light petroleum (40° to 60°)* until free from fatty material and dry at about 100°. Wash with a very small quantity of warm *chloroform* and dissolve the residue in 10 ml of a 1 per cent w/v solution of *sulphuric acid* (solution A). To 2 ml of the solution add one drop of *potassium mercuri-iodide solution*; a white precipitate is produced.

B. To 2 ml of the solution A add *sulphuric acid*; a reddish violet colour is produced.

C. Boil 2 ml of the solution A with a few drops of *nitric acid*; a yellow colour is produced. Cool and add *5 M sodium hydroxide*; the colour becomes yellowish brown.

Tests

Related substances. Determine by thin–layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of equal volumes of *butan–2–one* and *xylene.*

Test solution. Disperse a quantity of the suppositories containing about 20 mg of Bisacodyl with 20 ml of *petroleum spirit (boiling range,40° to 60°)*, filter, wash the residue with *petroleum spirit (boiling range, 40° to 60°)* until free from fat and dissolve in 2 ml of *acetone.*

Reference solution. Dilute 3.0 volumes of the test solution to 100.0 volumes with *acetone.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with a mixture of equal volumes of *0.05 M iodine* and *dilute sulphuric acid* and examine the plate in day light. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Suppositories.

Assay. Weigh a quantity of the suppositories containing about 50 mg of Bisacodyl, add 80 ml of *anhydrous glacial acetic acid* previously neutralised with *0.02 M perchloric acid* to *1-naphtholbenzein solution* and warm gently until solution is complete. Immediately titrate with *0.02 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.02 M perchloric acid* is equivalent to 0.007228 g of $C_{22}H_{19}NO_4$.

Storage. Store protected from light at a temperature not exceeding 30°.

Bisacodyl Gastro-resistant Tablets

Bisacodyl Tablets

Bisacodyl Gastro-resistant Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bisacodyl, $C_{22}H_{19}NO_4$. The tablets are rendered gastro-resistant by enteric coating or by other means.

Usual strength. 5 mg.

Identification

A. Extract a quantity of the powdered tablets containing 50 mg of Bisacodyl with *chloroform*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of a 1 per cent w/v solution of *sulphuric acid* (solution A). To 2 ml of the solution add one drop of *potassium mercuri-iodide solution*; a white precipitate is produced.

B. To 2 ml of solution A add *sulphuric acid*; a reddish-violet colour is produced.

C. Boil 2 ml of solution A with a few drops of *nitric acid*; a yellow colour is produced. Cool and add *5 M sodium hydroxide*; the colour becomes yellowish-brown.

Tests

Dissolution (2.5.2).

A. Apparatus No. 1 (Basket),
Medium. 500 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 120 minutes.

Withdraw a suitable volume of medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve about 50 mg of *bisacodyl IPRS* in 50 ml of *methanol*, add a drop of *orthophosphoric acid* and dilute with *0.1 M hydrochloric acid* to obtain 0.0005 per cent w/v solution of bisacodyl.

Chromatographic system
- a stainless steel column 10 cm × 4.0 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- column temperature: 40°,
- mobile phase: a mixture of 35 volumes of 0.1 per cent w/v solution of *ammonium acetate*, adjusted to pH 8.0 with *dilute ammonia solution* and 65 volumes of *acetonitrile*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{19}NO_4$.

Complies with the acceptance criteria given under acid stage.

After completion of A, remove the basket from the vessel and dip once into a 100 ml beaker containing 80 ml of *water*. After the *water* has drained from the basket, transfer the tablets to Apparatus No. 2 (Paddle) and carry out the procedure described under B.

B. Apparatus No. 2 (Paddle),

Medium. 900 ml of a buffer solution prepared by dissolving 8.9 g of *disodium hydrogen orthophosphate* and 10 g of *sodium lauryl sulphate* in 800 ml of *water*, adjusted the pH to 7.5 with *0.1 M hydrochloric acid* and diluted to 1000 ml with *water*.
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve about 50 mg of *bisacodyl IPRS* in 50 ml of *methanol* add a drop of *orthophosphoric acid* and dilute with dissolution medium to obtain a 0.00056 per cent w/v solution of bisacodyl.

Use chromatographic system as described under A.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{19}NO_4$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{19}NO_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 4 volumes of *glacial acetic acid,* 30 volumes of *acetonitrile* and 66 volumes of *water.*

Test solution. Disperse a quantity of the powdered tablets containing about 25 mg of Bisacodyl with 40 ml of the solvent mixture and dilute to 50.0 ml with the same solvent, filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *0.025 M ammonium formate,* previously adjusted to pH 5.0 with *anhydrous formic acid,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 50 µl.

Name	Relative retention time	Correction factor
Bisacodyl impurity A[1]	0.2	0.7
Bisacodyl impurity B[2]	0.4	---
Bisacodyl impurity C[3]	0.45	---
Bisacodyl impurity D[4]	0.8	---
Bisacodyl impurity E[5]	0.9	---
Bisacodyl (Retention time: about 13 minutes)	1.0	---
Bisacodyl impurity F[6]	2.6	---

[1]4,4′-(pyridin-2-ylmethylene)diphenol,

[2]2-[(RS)-(4-hydroxyphenyl)(pyridin-2-yl)methyl]phenol,

[3]4-[(RS)-(4-hydroxyphenyl)(pyridin-2-yl)methyl]phenyl acetate,

[4,6]unknown structure,

[5]2-[(RS)-[4-(acetyloxy) phenyl](pyridin-2-yl)methyl]phenyl acetate.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3.5 times of the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak corresponding to bisacodyl impurity C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent), the area of any secondary peak corresponding to bisacodyl impurity A is not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent), the area of any secondary peak corresponding to bisacodyl impurity E is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any secondary peak corresponding to bisacodyl impurity F is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any secondary peak corresponding to bisacodyl impurity D is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks excluding bisacodyl impurity A and C is not more than 5 times of the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using following test solution.

Test solution. Crush one tablet and disperse in 50 ml of the solvent mixture. Dilute 25.0 ml of the solution to 50.0 ml with the solvent mixture.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 4 volumes of *glacial acetic acid,* 30 volumes of *acetonitrile* and 66 volumes of *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 10 mg of Bisacodyl with 50 ml of the solvent mixture. Dilute 25.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *bisacodyl IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm) (such as Symmetry C18),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *0.025 M ammonium formate,* adjusted to pH 5.0 with *anhydrous formic acid,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{19}NO_4$ in the tablets.

Bismuth Subcarbonate

Bismuth Carbonate

$Bi_2(CO_3)O_2$ Mol. Wt. 510.0

Bismuth Subcarbonate contains not less than 80.0 per cent and not more than 82.5 per cent of Bi, calculated on the dried basis.

Category. Antacid.

Description. A white or almost white powder; odourless.

Identification

A. It gives the reactions of bismuth salts (2.3.1).

B. It gives reaction (A) of carbonates (2.3.1).

Tests

Appearance of solution. Disperse 5.0 g with 10 ml of *water*, add 20 ml of *nitric acid*. Heat to dissolve, cool and dilute to 100 ml with *water* (solution A). Solution A is not more opalescent than opalescence standard OS2 (2.4.1), and is colourless (2.4.1).

Alkalis and alkaline-earth metals. Not more than 1.0 per cent, determined by the following method. To 1.0 g add 10 ml of *water* and 10 ml of *5 M acetic acid*, boil for 2 minutes, cool, filter and wash the residue with 20 ml of *water*. To the combined filtrate and washings add 2 ml of *2 M hydrochloric acid* and 20 ml of *water*. Boil, pass hydrogen sulphide through the boiling solution until no further precipitate is produced, filter and wash the residue with *water*. Evaporate the combined filtrate and washings to dryness on a water-bath and add 0.5 ml of *sulphuric acid*, ignite gently and allow to cool.

Arsenic (2.3.10). To 0.5 g in a distillation flask add 5 ml of *water* and 7 ml of *sulphuric acid*, cool and add 5 g of *hydrazine reducing mixture* and 10 ml of *hydrochloric acid*. Connect the flask to an air-condenser, heat gradually to boiling during 15 to 30 minutes and continue heating at such a rate that the distillation proceeds steadily and until the volume in the flask is reduced by half, or until 5 minutes after the condenser has become full of steam. Discontinue distillation before fumes of sulphur trioxide are evolved. Collect the distillate in a tube containing 15 ml of *water* cooled in ice. Wash the condenser with *water* and dilute the combined distillate and washings to 25 ml with *water*. The resulting solution complies with the limit test for arsenic (5 ppm). Use 2.5 ml of *arsenic standard solution (1 ppm As)* diluted to 25 ml with *water* to prepare the standard.

Copper. To 5 ml of solution A add 2 ml of *10 M ammonia*, dilute to 50 ml with *water* and filter. To 10 ml of the filtrate add 1 ml of a 0.1 per cent w/v solution of *sodium diethyldithiocarbamate*. Any colour produced is not more intense than that produced by treating at the same time and in the same manner a solution containing 0.25 ml of *copper standard solution (10 ppm Cu)* diluted to 10 ml with *water* (50 ppm).

Lead. To 10 ml of solution A add 10 ml of *1 M sulphuric acid*; the solution does not become cloudy.

Silver. To 2.0 g add 1 ml of *water* and 4 ml of *nitric acid*. Heat gently to dissolve and dilute to 11 ml with *water*. Cool, add 2 ml of *1 M hydrochloric acid* and allow to stand for 5 minutes protected from light. Any opalescence produced is not more intense than that obtained by treating at the same time and in the same manner a mixture of 10 ml of *silver standard solution (5 ppm Ag)*, 2 ml of *1 M hydrochloric acid* and 1 ml of *nitric acid* (25 ppm).

Chlorides (2.3.12). To 10 ml of solution A add 4 ml of *nitric acid* and 20 ml of *water*; the resulting solution complies with the limit test for chlorides (500 ppm).

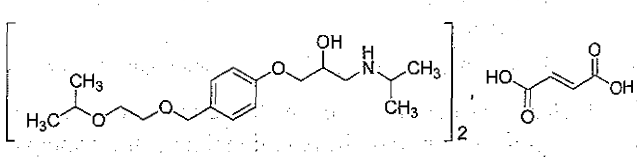
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.5 g, dissolve in 3 ml of *nitric acid* and dilute to 250 ml with *water*. Add *strong ammonia solution* until cloudiness is first observed, add 0.5 ml of *nitric acid* and heat to 70°, maintaining the solution at this temperature until the solution becomes completely clear. Add about 50 mg of *xylenol orange mixture* and titrate with *0.1 M disodium edetate* until the colour changes from pinkish-violet to lemon yellow.

1 ml of *0.1 M disodium edetate* is equivalent to 0.02090 g of Bi.

Storage. Store protected from light.

Bisoprolol Fumarate



$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$ Mol.Wt. 767.0

Bisoprolol Fumarate is 2-propanol,1-[4-[[2-(1-methylethoxy) ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-,(±)-,(*E*)-2-butenedioate.

Bisoprolol Fumarate contains not less than 97.5 per cent and not more than 102.0 per cent of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$, calculated on the anhydrous basis.

Category. Beta-adrenoceptor antagonist.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bisoprolol fumarate IPRS* or with the reference spectrum of bisoprolol fumarate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Optical rotation (2.4.22). – 2.0° to + 2.0°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay using the following modifications.

Inject the test solution. The sum of areas of all the secondary peaks, other than the peak due to fumaric acid is not more than 0.5 per cent, calculated by area normalization.

Fumaric acid. 14.8.per cent to 15.4 per cent.

Weigh 0.5 g and dissolve in 70 ml of *ethanol*, add 8.0 ml of *0.1M tetrabutylammonium hydroxide*, stir for 2 minutes. Continue to titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end point potentiometrically (2.4.25), using glass-calomel electrode. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.005804 g of fumaric acid.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 0.5 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 65 volumes of *water* and 35 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *bisoprolol fumarate IPRS* in the solvent.

Reference solution (b). A solution containing 0.05 per cent w/v of *propranolol hydrochloride IPRS* and 0.1 per cent w/v of *bisoprolol fumarate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: To 1000 ml of solvent mixture, add 5 ml of *heptafluorobutyric acid*, 5 ml of *diethylamine* and 2.5 ml of *formic acid*. Mix and filter, make necessary adjustment if necessary to obtain desired resolution.
- flow rate: 1 ml per minute,
- spectrophotometer set at 273 nm,
- injection volume: 10 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to bisoprolol and propranalol is not less than 7 in the chromatogram obtained with reference solution (b), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for bisoprolol peak in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$ using the area of the 2 major peaks.

Storage. Store protected from light and moisture at a temperature below 30°.

Bisoprolol Fumarate and Hydrochlorothiazide Tablets

Bisoprolol Fumarate and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of bisoprolol fumarate, $(C_{18}H_{31}NO_4)_2$, $C_4H_4O_4$ and hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

Usual strengths. Bisoprolol Fumarate, 2.5 mg, Hydrochlorothiazide, 6.25 mg; Bisoprolol Fumarate, 5 mg, Hydrochlorothiazide, 6.25 mg; Bisoprolol Fumarate 10 mg, Hydrochlorothiazide 6.25 mg.

Identification

A. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 43 volumes of *dichloromethane*, 20 volumes of *methanol* and 8 volumes of *strong ammonia solution*.

Test solution. Disperse 1 tablet in 5-ml volumetric flask. Dilute with *methanol* to volume, sonicate for 5 minutes and centrifuge and use the clear supernatant liquid.

Reference solution (a). A 0.1 per cent w/v solution of *bisoprolol fumarate IPRS* in *methanol*.

Reference solution (b). A 0.1 per cent w/v solution of *hydrochlorothiazide IPRS* in *methanol*.

Apply to the plate 25 µl of each solution. After development, dry the plate in current of air and examine under ultraviolet light at 254 nm as well as by exposure to iodine vapour. The two principal spots in the chromatogram obtained with test solution corresponds to those in the chromatogram obtained with reference solution (a) and (b).

B. In the Assay, the principal peaks in the chromatogram obtained with test solution (a) corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 75 rpm and 20 minutes for bisoprolol fumarate and 30 minutes for hydrochlorothiazide.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution (a). A 0.05 per cent w/v solution of *bisoprolol fumarate IPRS* in dissolution medium.

Reference solution (b). Weigh 30 mg of *hydrochlorothiazide IPRS* in 50-ml volumetric flask, dissolve in 5 ml of *methanol* and dilute to volume with dissolution medium.

Reference solution (c). Dilute a volume of reference solution (a) and reference solution (b) with dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with phenyl groups bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *triethylamine solution* prepared by mixing 2 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *ortho-phosphoric acid* and 10 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 227 nm for bisoprolol fumarate and 272 nm for hydrochlorothiazide,
- injection volume: 20 µl.

Inject reference solution (c) and the test solution.

Q. Not less than 80 per cent of the stated amount of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$ and $C_7H_8ClN_3O_4S_2$.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay with the following modifications.

Test solution (a). Weigh and powder 10 tablets and transfer in to 100-ml volumetric flask. Add 50 ml of solvent mixture, sonicate for 10 minutes, and cool. Dilute with solvent mixture up to volume, stir by mechanical means for 1 hour, and centrifuge.

Test solution (b). Dilute a suitable volume of test solution (a) with the solvent mixture to obtain a solution containing 0.01 per cent w/v of bisoprolol fumarate.

Reference solution. A solution containing 0.0002 per cent w/v of *hydrochlorothiazide IPRS* in solvent mixture.

Chromatographic system

- spectrophotometer set at 260 nm,

The correction factor 0.83 for the peak with a relative retention time of 0.69 and 0.71 for the peak with a relative retention time 1.2 with reference to hydrochlorothiazide.

Inject the reference solution and test solution (b). In the chromatogram obtained with test solution (b) the area of any peak at relative retention time of 1.2 is not more than the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). The area of any other secondary peak at relative retention time of 0.69 is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Calculate the percentage of each impurity using following expression:

$$100 \times C \times \frac{Lb}{Lh} \times \frac{Cs}{Cb} \times \frac{Rt}{Rs}$$

where, C = Correction factor for the peak with relative retention time of 0.69 and 1.2,

Lb = Labelled quantity of bisoprolol fumarate in mg,

Lh = Labelled quantity of hydrochlorothiazide in mg,

Cs = Concentration of *hydrochlorothiazide IPRS* in reference solution (mg per ml),

Cb = Concentration of bisoprolol fumarate in test solution (b) (mg per ml),

Rt = Peak area of each of the two impurity obtained from test solution (b),

Rs = Peak area of hydrochlorothiazide peak obtained from reference solution.

Uniformity of content. Complies with the test stated under tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Transfer one tablet in 25-ml volumetric flask. Add 12.5 ml of solvent mixture, sonicate for 10 minutes, and cool. Dilute with solvent mixture to volume, stir by mechanical means for 1 hour, and centrifuge. Dilute further if necessary, with the solvent mixture.

Inject reference solution (b) and the test solution.

Calculate the content of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$ and $C_7H_8ClN_3O_4S_2$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dilute 10 ml of *1 M dibutylammonium phosphate* with 1000 ml of a mixture prepared by mixing 50 volumes of *water* and 50 volumes of *acetonitrile.*

Test solution (a). Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 25 mg of Bisoprolol Fumarate in 100-ml volumetric flask and dilute to volume with the solvent mixture.

Test solution (b). Dilute a suitable volume of test solution (a) with the solvent mixture to obtain a solution containing 0.01 per cent w/v of bisoprolol fumarate.

Test solution (c). Dilute a suitable volume of test solution (a) with solvent mixture to obtain a solution containing 0.00625 per cent w/v of hydrochlorothiazide.

Reference solution (a). A solution containing 0.004 per cent w/v each of *chlorothiazide IPRS and hydrochlorothiazide IPRS in solvent mixture.*

Reference solution (b). A solution containing 0.01 per cent w/v of *bisoprolol fumarate IPRS* and 0.00625 per cent w/v of *hydrochlorothiazide IPRS* in solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 8.0 mm, packed with phenyl groups bonded to porous silica (5 µm),
- mobile phase: A. dilute 10 ml of *1 M dibutylammonium phosphate* with 1000 ml of *water,*
  B. a mixture of 30 volumes of *acetonitrile* and 20 volumes of *water,* add 0.5 volume of *1 M dibutyl ammonium phosphate* and stir vigorously for 2 minutes, filter.
- a gradient programme using the conditions given below,
- flow rate: 3 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
9	40	60
9.1	100	0
12	100	0

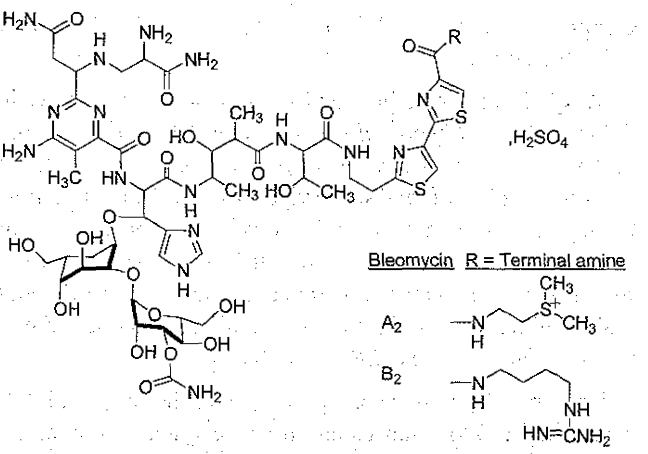
Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to chlorothiazide and hydrochlorothiazide is not less than 1.5 obtained with reference solution (a), the tailing factor due to hydrochlorothiazide is not more than 1.3 obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and test solution (b), (c).

Calculate the content of $(C_{18}H_{31}NO_4)_2,C_4H_4O_4$ and $C_7H_8ClN_3O_4S_2$ in the tablets.

Storage. Store protected from light and moisture.

Bleomycin Sulphate



$C_{55}H_{84}N_{17}O_{21}S_3,H_2SO_4$ Mol. Wt. 1513.6
(Bleomycin A_2 Sulphate)

$C_{55}H_{84}N_{20}O_{21}S_2,H_2SO_4$ Mol. Wt. 1523.6
(Bleomycin B_2 Sulphate)

Bleomycin Sulphate is the sulphate salt of bleomycin, a mixture of basic cytotoxic glycopeptides produced by the growth of *Streptomyces verticillus* or produced by other means. Its main components are bleomycin A_2 and bleomycin B_2. Bleomycin A_2 sulphate is N^1-[3 (dimethylsulphonio)propyl]bleomycina-mide hydrogen sulphate and Bleomycin B_2 is N^1-(guanidinobutyl) bleomycinamide sulphate.

Bleomycin Sulphate contains not less than 1.5 and not more than 2.0 Units of bleomycin per mg and the content of bleomycins is: bleomycin A_2, between 55 per cent and 70 per cent;bleomycin B_2, between 25 per cent and 32 per cent; sum of bleomycin A_2 and bleomycin B_2, not less than 85 per cent; demethylbleomycin A_2, not more than 5.5 per cent; other related substances, not more than 9.5 per cent.

Category. Anticancer.

Description. A white or cream-coloured, amorphous powder.

CAUTION — Bleomycin Sulphate must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bleomycin sulphate IPRS* or with the reference spectrum of bleomycin sulphate.

B. It gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.0, determined in a solution containing 10 Units per ml.

Copper. Not more than 0.02 per cent determined by Method A or by Method B.

A. Weigh 50 mg, transfer to a 60-ml separator and dissolve in 10.0 ml of *0.1 M hydrochloric acid*. Add 10 ml of a 0.01 per cent w/v solution of *zinc bis (diphenyl dithiocarbamate)* in *carbon tetrachloride* and shake vigorously for 1 minute. Allow the layers to separate, filter the lower layer through 1 g of *anhydrous sodium sulphate*. Treat similarly 1.0 ml of *copper standard solution (10 ppm Cu)* and measure the absorbances (2.4.7) of the two solutions at the maximum at about 435 nm, using *carbon tetrachloride* as the blank.

B. Determine by atomic absorption spectrophotometry (2.4.2) measuring at 324.7 nm using an air-acetylene flame and a solution prepared by dissolving 50 mg of the substance under examination in *water* and dilute to 10.0 ml with *water*. Use *copper solution AAS* suitably diluted with *water*, for preparing the reference solutions.

Content of bleomycins. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve the substance under examination in freshly boiled and cooled *water* so as to give a solution containing about 2.5 Units per ml. (*The solution should be stored at 2° to 8° just before use*).

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: Transfer 0.96 g of *sodium 1-pentanesulphonate* to a 1000-ml volumetric flask, add 5.0 ml of *glacial acetic acid* and 900 volumes of *water*. Mix and adjusted to pH 4.3 with *strong ammonia solution* (1.86 g of *disodium edetate* may be included if needed for satisfactory chromatography). Adjust the volume with *water*, mix well, filter and degas before use. Use a linear gradient of 10 per cent to 40 per cent *methanol*, which also is filtered and degassed before use, mixed with the solution,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

After the final conditions are reached (about 60 minutes) allow the chromatography to proceed with the final gradient mixture for an additional 20 minutes or until demethylbleomycin A_2 is eluted.

Inject the test solution and proceed with gradient elution, pumping the mobile phase mixture under the conditions mentioned above for about 80 minutes or until the demethylbleomycin A_2 is eluted. The usual order of elution is bleomycinic acid, bleomycin A_2 (first principal peak), bleomycin A_5, bleomycin B_2 (second principal peak), bleomycin B_4 and demethylbleomycin A_2 (retention time relative to bleomycin A_2, between 1.5 and 2.5).

Measure the peak responses of all peaks. Calculate the contents of each bleomycin component by comparing the ratios of the individual areas of the peaks with that of the total area of all bleomycins.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 50 mg by drying in an oven over *phosphorous pentoxide* at 60° at a pressure not exceeding 0.25 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10), and express the result in Units per mg.

Bleomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 10.0 Endotoxin Units per unit of bleomycin.

Bleomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. If the material is sterile, it should be stored in sterile, tamper-evident containers and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the strength with respect to Bleomycin Sulphate as the number of bleomycin Units per mg; (2) whether or not the contents are intended for use in the manufacture of parenteral preparations.

Bleomycin Injection

Bleomycin Sulphate Injection

Bleomycin Injection is a sterile freeze dried material consisting of Bleomycin Sulphate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of the liquid stated on the label before use.

The constituted solution complies with the tests for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Bleomycin injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of bleomycin and the content of bleomycins is: bleomycin A_2, between 55 per cent and 70 per cent;bleomycin B_2, between 25 and 32 per cent; sum of bleomycin A_2 and bleomycin B_2, not less than 85 per cent; demethylbleomycin A_2, not more than 5.5 per cent; other related substances, not more than 9.5 per cent.

The contents of the sealed container comply with the tests stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strengths. 15 units per vial; 30 units per vial.

Identification

A. Determine by infra-red absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bleomycin sulphate IPRS* or with the reference spectrum of bleomycin sulphate.

B. It gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.0, determined in a solution containing 10 Units per ml.

Copper. Not more than 0.02 per cent, determined by Method A or Method B

A. Weigh a quantity containing about 50 mg of bleomycin, transfer to a 60-ml separator and dissolve in 10.0 ml of *0.1 M hydrochloric acid*. Add 10 ml of a 0.01 per cent w/v solution of *zinc bis(diphenyl dithiocarbamate)* in *carbon tetrachloride* and shake vigorously for 1 minute. Allow the layers to separate, filter the lower layer through 1 g of *anhydrous sodium sulphate*. Treat similarly 1.0 ml of *copper standard solution (10 ppm Cu)* and measure the absorbances (2.4.7) of the two solutions at the maximum at about 435 nm, using *carbon tetrachloride* as the blank.

B. Determine by atomic absorption spectrophotometry (2.4.2) measuring at 324.7 nm using an air-acetylene flame and a solution prepared in the following manner: Weigh a quantity containing about 75 mg of bleomycin, dissolve in *water* and dilute to 10 .0 ml with the same solvent. Use *copper solution AAS* suitably diluted with *water*, for preparing the reference solutions.

Content of bleomycin. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a suitable quantity dissolve in freshly boiled and cooled *water* and dilute to obtain a solution containing about 2.5 Units per ml. (*The solution should be stored at 2° to 8° just before use*).

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: Transfer 0.96 g of *sodium 1-pentanesulphonate* to a 1000-ml volumetric flask, add 5.0 ml of *glacial acetic acid* and 900 volumes of *water*. Mix and adjusted to pH 4.3 with *strong ammonia solution* (1.86 g of *disodium edetate* may be included if needed for satisfactory chromatography). Adjust the volume with *water*, mix well, filter and degas before use. Use a linear gradient of 10 per cent to 40 per cent *methanol*, which also is filtered and degassed before use, mixed with the solution,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

After the final conditions are reached (about 60 minutes) allow the chromatography to proceed with the final gradient mixture for an additional 20 minutes or until demethylbleomycin A_2 is eluted.

Inject the test solution and proceed with gradient elution, pumping the mobile phase mixture under the conditions mentioned above for about 80 minutes or until the demethylbleomycin A_2 is eluted. The usual order of elution is bleomycinic acid, bleomycin A_2 (first principal peak), bleomycin A_5, bleomycin B_2 (second principal peak), bleomycin B_4 and demethylbleomycin A_2 (retention time relative to bleomycin A_2, between 1.5 and 2.5).

Measure the peak responses of all the peaks. Calculate the contents of each bleomycin component by comparing the ratios of the individual areas of the peaks with that of the total area of all bleomycins.

Bacterial endotoxins (2.2.3). Not more than 10.0 Endotoxin Units per unit of bleomycin.

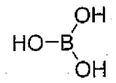
Loss on drying (2.4.19). Not more than 6.0 per cent, determined by drying the combined contents of two containers in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine the weight of the contents of 10 containers. Mix the contents of the containers and determine by the microbiological assay of antibiotics, Method A or B (2.2.10) and express the results in Units per vial.

Storage. The sealed container should be protected from light.

Labelling. The label states the total number of units contained in the sealed container.

Boric Acid



H_3BO_3 Mol. Wt. 61.8

Boric Acid contains not less than 99.5 per cent and not more than 100.5 per cent of H_3BO_3, calculated on the dried basis.

Category. Local anti-infective.

Description. A white, crystalline powder or colourless shiny plates unctuous to the touch or white crystals; odourless.

Identification

A. Dissolve 0.1 g by gently warming with 5 ml of *methanol* to which a few drops of *sulphuric acid* have been added. Ignite the solution; the flame has a green border.

B. Dissolve 3.0 g in 90 ml of boiling *distilled water*, cool; the solution is slightly acid (2.4.46).

Tests

Appearance of solution. A 3.5 per cent w/v solution in boiling *water* is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 3.8 to 4.8, determined in the solution obtained in Identification test B.

Solubility in ethanol. Dissolve 1.0 g in 10 ml of boiling *ethanol (95 per cent)*; the solution is not more opalescent than opalescence standard OS2 (2.4.1), and colourless (2.4.1).

Arsenic (2.3.10). Dissolve 1.0 g in 50 ml of *water* containing 2 g of *citric acid* and add 0.1 ml of *stannous chloride solution AsT* and 10 ml of *hydrochloric acid*. The resulting solution complies with the limit test for arsenic (10 ppm).

Heavy metals (2.3.13). A solution produced by dissolving 1.0 g in 2 ml of *dilute acetic acid* and diluting with sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

Sulphates (2.3.17). Dissolve 0.33 g in 10 ml of boiling *water* and dilute to 15 ml with *water*. The solution complies with the limit test for sulphates (450 ppm).

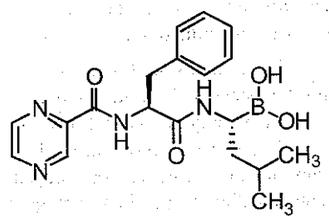
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *silica gel* for 5 hours.

Assay. Weigh 2.0 g, dissolve in a mixture of 50 ml of *water* and 100 ml of *glycerin*, previously neutralised to *phenolphthalein solution*. Titrate with *1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.06183 g of H_3BO_3.

Labelling. The label states that it is not meant for internal use.

Bortezomib



$C_{19}H_{25}BN_4O_4$ Mol. Wt. 384.2

Bortezomib is [(1*R*)-3-methyl-1-[[(2*S*)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl)-amino]propyl]amino]butyl]boronic acid.

Bortezomib contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{25}BN_4O_4$, calculated on the dried basis.

Category. Anticancer.

Description. A white to off-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bortezomib IPRS* or with the reference spectrum of bortezomib.

Tests

Specific Optical Rotation (2.4.22). −55.0° to −45.0°, calculated on dried basis and determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 5.0 ml of mobile phase B and dilute to 50.0 ml with mobile phase A.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- sample temperature: 5°,
- mobile phase: A. a mixture of 30 volumes of *acetonitrile*, 70 volumes of *water* and 0.1 volume of *formic acid*,
  B. a mixture of 80 volumes of *acetonitrile*, 20 volumes of *water* and 0.1 volume of *formic acid*,
- a gradient programme using the conditions given below,

- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
15	100	0
30	0	100
45	0	100
47	100	0
55	100	0

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Chiral purity. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

The retention time of bortezomib peak is about 8.5 minutes and of S, S enantiomer is about 10.5 minutes.

Inject the test solution. The area of the peak corresponding to S, S enantiomer is not more than 0.5 per cent, calculated by area normalization.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by drying over *phosphorus pentoxide* at room temperature, under vacuum at a pressure of 1.5kPa to 2.5kPa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances with the following modifications.

Test solution. Dissolve 25 mg of the substance under examination in 5 ml of mobile phase B and dilute to 50.0 ml with mobile phase A.

Reference solution. Dissolve 25 mg of *bortezomib IPRS* in 5 ml of mobile phase B and dilute to 50.0 ml with mobile phase A.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{25}BN_4O_4$.

Bortezomib Injection

Bortezomib Injection is a sterile freeze dried material consisting of Bortezomib with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of *0.9 per cent w/v sodium chloride injection* immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Bortezomib injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bortezomib $C_{19}H_{25}BN_4O_4$.

Usual strengths. 2 mg per vial; 3.5 mg per vial

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements stated under Parenteral preparations (Powders for injection) and with the following requirements.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH. (2.4.24). 4.0 to 7.0, when constituted as 1 mg per ml with 0.9 per cent w/v *sodium chloride injection*.

Appearance of solution. A constituted solution containing 1 mg of bortezomib per ml with 0.9 per cent w/v *sodium chloride injection* is clear (2.4.1) and colourless (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of 0.9 per cent w/v of *sodium chloride* and 70 volumes of *acetonitrile*.

Test solution. Determine the weight of the content of 10 containers. Disperse a quantity of the mixed contents of the 10 containers containing 10 mg of Bortezomib, dissolve in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm)
- mobile phase: A. a mixture of 1000 volumes of *water* and 1 volume of *formic acid*,
  B. *acetonitrile*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
15	70	30
30	40	60
60	40	60
65	70	30
70	70	30

Inject the test solution. The area of any secondary peak is not more than 1.0 per cent and the sum of the areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalisation.

Tertiary Butanol (*If present*). Not more than 5000 ppm.

Determine by gas chromatography (2.4.13).

Test solution. Transfer mixed contents of the containers containing 0.025 g of bortezomib to a 20 ml vial, add 1.0 ml of *dimethylformamide*.

Reference solution (a). A 0.025 per cent w/v solution of *tertiary butanol* in *dimethylformamide*.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 10.0 ml with *dimethylformamide*. Transfer 1.0 ml of the solution to a 20.0 ml vial.

Chromatographic system

- a capillary column 30 m x 0.53 mm, packed with bonded and crosslinked siloxane (3 µm),
- temperature:

column	time (in min.)	temperature (°)
	0 – 8	40
	8 – 10	80
	10 – 14.5	90

- inlet port at 200° and detector at 250°,
- flame ionization detector,
- flow rate: 4 ml per minute, helium as the carrier gas.

Inject 1 µl of vapour phase from the reference solution (b) and the test solution.

Calculate the content of tertiary butanol from the peak responses of tertiary butanol in the test solution and the reference solution (b).

Bacterial endotoxins (2.2.3). Not more than 75 Endotoxin Units per mg of bortezomib.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 35 volumes of *acetonitrile*, 65 volumes of *water* and 0.1 volume of *formic acid*.

Test solution: Determine the weight of the content of 10 containers. Weigh a quantity of the mixed contents of the 10 containers containing 20 mg of Bortezomib, dissolve in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.04 per cent w/v solution of *bortezomib IPRS* in the solvent mixture.

Chromatographic system:

- a stainless steel column 15 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *acetonitrile*, 70 volumes of *water* and 0.1 volume of *formic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

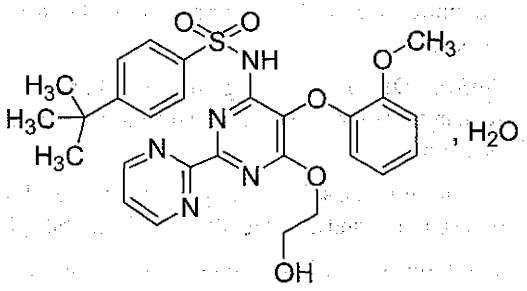
Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{25}BN_4O_4$ in the injection.

Storage. Store protected from light and moisture.

Labeling. The label states the quantity of bortezomib contained in the sealed container.

Bosentan Monohydrate



$C_{27}H_{29}N_5O_6S,H_2O$ Mol. Wt. 569.6

Bosentan Monohydrate is 4-(1, 1- Dimethylethyl)-*N*-[6-(2-hydroxyethoxy)-5-(2- methoxyphenoxy)[2,2-bipyrimidin]-4-yl] benzenesulfonamide monohydrate.

Bosentan Monohydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{27}H_{29}N_5O_6S$, calculated on the anhydrous basis.

Category. Antihypertensive.

Description. A white to yellowish powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bosentan monohydrate IPRS* or with the reference spectrum of bosentan monohydrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture A. a mixture of 90 volumes of *acetonitrile* and 10 volumes of *water*.

Solvent mixture B. a mixture of equal volumes of mobile phase A and mobile phase B.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture B and dilute to 50.0 ml with the solvent mixture B.

Reference solution. A 0.01 per cent w/v solution of *bosentan monohydrate IPRS* in the solvent mixture A. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture B.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl silica gel bonded to porous silica (5 µm), (Such as Zorbax SB-Phenyl),
- sample temperature: 8°,
- column temperature: 35°,
- mobile phase: A. a mixture of 40 volumes of *methanol* and 60 volumes of a buffer solution prepared by diluting 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
5	70	30
25	40	60
30	40	60
35	70	30
40	70	30

Name	Relative retention time	Correction factor
Bosentan related compound E[1]	0.39	1.15
Bosentan related compound D[2]	0.57	1.38
Bosentan related compound B[3]	0.96	0.99
Bosentan	1.0	---
Bosentan related compound A[4]	1.34	1.16
Bosentan related compound C[5]	2.15	1.12

[1]4-(tert-butyl) benzensulfonamide,

[2]4,6-Dichloro-5-(2-methoxyphenoxy)-2,2'-bipyrimidine,

[3]4-(tert-butyl)-N-[6-hydroxy-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl] benzensulfonamide,

[4]4-(tert-butyl)-N-[6-chloro-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl benzensulfonamide,

[5]1,2-Bis({6-[4-(tert-butyl)phenylsulfonamido]-5-(2-methoxyphenoxy)-[2,2'-bipyrimidine]-4-yl}oxy) ethane.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with test solution, the area of any peak due to bosentan related compounds A, B, C, D and E is not more than 1.5 times the area of principal peak in the chromatogram obtained with the reference solution (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not less than 2.0 per cent and not more than 4.0 per cent, determined on 0.1g dissolved in a mixture of equal volumes of *methanol* and *dimethylformamide*.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *bosentan monohydrate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl silica gel bonded to porous silica (5 µm), (Such as Zorbax SB-Phenyl),

- sample temperature: 8°,
- column temperature: 35°,
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of a buffer solution prepared by diluting 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{29}N_5O_6S$.

Storage. Store in tight container and at a temperature not exceeding 25°.

Bosentan Tablets

Bosentan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bosentan, $C_{27}H_{29}N_5O_6S$.

Usual strengths. 62.5 mg; 125 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a buffer solution prepared by dissolving 6.8 g *sodium dihydrogen orthophosphate* in 1000 ml of *water* and mix. Adjusted to pH 6.8 with 10 per cent w/v solution of *sodium hydroxide*. To the solution add 5 g of *sodium lauryl sulphate*,

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, Dilute if necessary, with the mobile phase.

Reference solution. Dissolve a quantity of *bosentan monohydrate IPRS* in *methanol* and dilute with the mobile phase to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Zorbax SB- C18),
- column temperature: 40°,
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water* and 70 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 10 µl.

Inject the reference solution and the test solution.

Q. Not less than 75 per cent of the stated amount of $C_{27}H_{29}N_5O_6S$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. a mixture of equal volumes of *acetonitrile* and a buffer solution prepared by diluting 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted pH 3.0 with *orthophosphoric acid.*

Test solution. Disperse an accurately weighed quantity of the tablet powder containing 50 mg of bosentan in 30 ml of the solvent mixture with the aid of ultrasound for 10 minutes and dilute to 50.0 ml with the solvent mixture, mix well and filter.

Reference solution . A 0.001 per cent w/v solution of *bosentan monohydrate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS 3V),
- column temperature: 40°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by diluting 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 45 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with test solution, the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution

(2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse an accurately weighed quantity of the powder containing 125 mg of bosentan in 140 ml of the *methanol* with the aid of ultrasound for 10 minutes and dilute to 200.0 ml with the *methanol*, mix well and filter. Dilute 5.0 ml of the solution to 25.0 ml with the *methanol*.

Reference solution. A 0.0125 per cent w/v solution of *bosentan monohydrate IPRS* in *methanol*.

Use chromatographic system as described under Dissolution.

Inject 20 µl of the reference solution and the test solution.

Inject the reference solution. The test is not valid unless the column efficiency in not less than 800 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

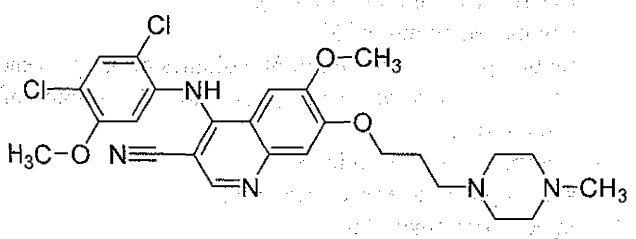
Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{29}N_5O_6S$ in the tablets.

Storage. Store in a cool and dry place.

Labelling. The label states the strength in terms of the equivalent amount of bosentan.

Bosutinib



$C_{26}H_{29}Cl_2N_5O_3$ Mol. Wt. 530.5 (anhydrous form)

Bosutinib is 3-Quinolinecarbonitrile,4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl) propoxy].

Bosutinib contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{26}H_{29}Cl_2N_5O_3$, calculated on anhydrous basis.

CAUTION — Bosutinib is cytotoxic, extra care required to prevent inhaling particles and exposing the skin to it.

Category. Anticancer

Description. A white to yellowish-tan powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bosutinib IPRS* or with the reference spectrum of bosutinib.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *acetonitrile* and 30 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.01 per cent w/v solution of *bosutinib IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (Such as YMC Triart),
- mobile phase: A. a buffer solution prepared by dissolving 1.74 g of *dipotassium hydrogen ortho-phosphate* in 1000 ml of *water*, adjusted to pH 10.0 with 1per cent w/v solution of *potassium hydroxide*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate:1 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
10	35	65
35	35	65
37	75	25
45	75	25

Inject reference solution (a) and (b). The test is not valid unless the column efficiency is not less than 22000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard

deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (a) and the signal-to-noise ratio is not less than 10 in the chromatogram obtained with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

*Solvent mixture.*70 volumes of *acetonitrile* and 30 volumes of *water.*

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *bosutinib IPRS* in the solvent mixture.

Chromatographicsystem
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as X-Bridge C18),
- column temperature: 40°,
- sample temperature:10°,
- mobile phase: a mixture of 50 volumes of 0.1 per cent v/v of *triethylamine* in *water* and 50 volumes of *acetonitrile,*
- flow rate:1 ml per minute,
- spectrophotometer set at 269 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution. Calculate the content of $C_{26}H_{29}Cl_2N_5O_3$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Bosutinib Tablets

Bosutinib Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bosutinib, $C_{26}H_{29}Cl_2N_5O_3$.

Usual strengths. 100 mg; 400 mg; 500 mg.

CAUTION — Bosutinib is cytotoxic, extra care required to prevent inhaling particles and exposing the skin to it.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *bosutinib IPRS* in the dissolution medium and dilute with the dissolution medium to obtain a solution containing 0.011 per cent w/v of bosutinib.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Kromasil Eternity XT C-18),
- column temperature: 35°,
- mobile phase: a mixture of 58 volumes of 0.1 per cent v/v of *triethylamine* in *water* and 42 volumes of acetonitrile,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 269 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{29}Cl_2N_5O_3$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{26}H_{29}Cl_2N_5O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water.*

Test solution. Disperse a quantity of the powdered tablets containing 100 mg of Bosutinib in the solvent mixture, with the aid of mechanical shaker for 30 minutes, dilute to 200.0 ml with the solvent mixture, mix and filter.

Reference solution (a). A 0.0005 per cent w/v solution of *bosutinib IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 20.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as YMC Triart),
- column temperature: 45°,
- sample temperature: 5°,
- mobile phase: A. a mixture of 95 volumes of a buffer solution prepared by dissolving 3.08 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 6.8 with *ammonia solution* and 5 volumes of *acetonitrile*,
  B. a mixture of 90 volumes of *acetonitrile* and 10 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
25	45	55
33	35	65
40	20	80
45	20	80
45.5	65	35
52	65	35

Inject reference solution (a) and (b). The test is not valid unless the column efficiency is not less than 12000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (a) and the signal-to-noise ratio is not less than 10 in the chromatogram obtained with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *acetonitrile* and 30 volumes of *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 250 mg of Bosutinib in the solvent mixture, with the aid of mechanical shaker for 30 minutes and dilute to 250.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 20.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *bosutinib IPRS* in the solvent mixture.

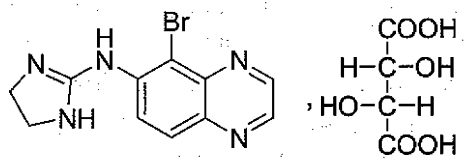
Use chromatographic system as described under dissolution.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. Calculate the content of $C_{26}H_{29}Cl_2N_5O_3$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Brimonidine Tartrate



$C_{11}H_{10}BrN_5, C_4H_6O_6$ Mol. Wt. 442.2

Brimonidine Tartrate is 5-bromo-6-(2-imidazolidinylidene-amino) quinoxaline *L*-tartrate.

Brimonidine Tartrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{11}H_{10}BrN_5, C_4H_6O_6$, calculated on dried basis

Category. Antiglaucoma.

Description. A white to slightly yellowish powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *brimonidine tartrate IPRS* or with the reference spectrum of brimonidine tartrate.

Tests

pH (2.4.24). 3.0 to 4.5, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). +9.0° to + 10.5°, determined in a 1.0 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution. A 0.00005 per cent w/v solution of *brimonidine tartrate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of *0.01M triethylamine*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.25 g in 40 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.04422 g of $C_{11}H_{10}BrN_5, C_4H_6O_6$.

Storage. Store protected from light and moisture.

Brimonidine Tartrate Eye Drops

Brimonidine Tartrate Eye Drops is a sterile solution of Brimonidine tartrate in Purified Water.

Brimonidine Tartrate Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of brimonidine tartrate, $C_{11}H_{10}BrN_5, C_4H_6O_6$.

Usual strengths. 0.15 per cent w/v; 0.2 per cent w/v.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.7 to 8.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the eye drops containing 10 mg of Brimonidine Tartrate to 20.0 ml with the mobile phase.

Reference solution. A 0.0005 per cent w/v solution of *brimonidine tartrate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 mm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of buffer solution prepared by diluting 1.4 ml of *triethylamine* in 1000 ml *water*, adjusted to pH 7.2 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume: 10 µl.

The relative retention time with reference to brimonidine tartrate for debromobrimonidine impurity is about 0.6 and correction factor for debromobrimonidine impurity is 2.77.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to debromobrimonidine impurity is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The area of any other secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent). The sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the eye drops containing 20 mg of Brimonidine Tartrate to 250.0 ml with the mobile phase.

Reference solution. A 0.008 per cent w/v solution of *brimonidine tartrate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of buffer solution prepared by diluting 1.4 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH to 7.2 with *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume: 10 µl.

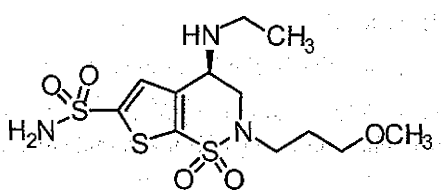
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{10}BrN_5$, $C_4H_6O_6$ in the Eye Drops.

Storage. Store protected from light.

Brinzolamide



$C_{12}H_{21}N_3O_5S_3$ Mol Wt. 383.5

Brinzolamide is(*R*)-4-(ethylamino)-3,4-dihydro-2-(3-methoxypropyl)-2*H*-thieno[3,2-e]-1,2-thiazine-6-sulphonamide1,1-dioxide.

Brinzolamide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{21}N_3O_5S_3$, calculated on the dried basis.

Category. Antiglaucoma agent.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *brinzolamide IPRS* or with the reference spectrum of brinzolamide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Limit of Brinzolamide Related Compound A

Test solution. Dissolve about 25 mg of the substance under examination in *ethanol* and dilute to 50.0 ml with *ethanol.*

Reference solution. A solution containing 0.04 per cent w/v of *brinzolamide IPRS* and 0.002 per cent w/v of *brinzolamide impurity A IPRS (S-(-)-4-ethylamino-2, 3-dihydro-2-(-3-methoxypropyl)-4H-thieno-[3,2,e]-thiazine-6-sulphonamide-1,1-dioxide)* in *ethanol.*

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, such as chiral Pack ADH (5 µm),
- mobile phase: a mixture of 55 volumes of *ethanol,* 40 volumes of *hexane,* 5 volumes of *methanol* and 0.2 volume of *diethylamine,*
- flow rate: 0.75 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5 µl.

The relative retention time with reference to brinzolamide for brinzolamide impurity A is about 1.2.

Inject the reference solution. The test is not valid unless the resolution between brinzolamide and brinzolamide impurity A is not less than 1.8, the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 1.8 for the brinzolamide peak.

Inject the test solution. The area of any peak due to brinzolamide impurity A is not more than 0.5 per cent, calculated by area normalization.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution. A solution containing 0.01 per cent w/v each of *brinzolamide IPRS* and *brinzolamide impurity B IPRS ((R)-4-Amino-2-(3-methoxypropyl)-3,4-dihydro-2H-thieno[3,2-e][1,2]thiazine-6-sulphonamide 1,1-dioxide oxalate* in mobile phase A.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 75 volumes of a buffer solution prepared by dissolving 4.0 ml of *triethylamine* in 1000 ml of *water* adjusted to pH 3.0 with *ortho-phosphoric acid* and 25 volumes of *acetonitrile,*

B. a mixture of 65 volumes of a buffer solution prepared by dissolving 4.0 ml of *triethylamine* in 1000 ml of *water* adjusted to pH 3.0 with *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

The relative retention time with reference to brinzolamide for brinzolamide impurity B is about 0.8 using mobile phase A.

Inject the reference solution. The test is not valid unless the resolution between brinzolamide and brinzolamide impurity B is not less than 2.0, the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0 for the brinzolamide peak.

Analysis 1

Inject the test solution using mobile phase A. Run the chromatogram for about 20 minutes. The area of any secondary peak is not more than 0.3 per cent, calculated by area normalization.

Analysis 2

Inject the test solution using mobile phase B. Run the chromatogram for about 20 minutes and measure the areas for brinzolamide and all the peaks having a relative retention not less than 6.

The area of any secondary peak is not more than 0.3 per cent and the sum of areas of all secondary peaks is not more than 1.0 per cent, calculated by area normalization from analysis1 and analysis 2.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *brinzolamide IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of buffer solution prepared by dissolving 4.0 ml of *triethylamine* in 1000 ml of *water* adjusted to pH 3.0 with *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the the column efficiency is not less than 1200 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{12}H_{21}N_3O_5S_3$.

Brinzolamide Ophthalmic Suspension

Brinzolamide Ophthalmic Suspension is a sterile, aqueous suspension of Brinzolamide containing a suitable antimicrobial preservative.

Brinzolamide Ophthalmic Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of brinzolamide, $C_{12}H_{21}N_3O_5S_3$.

Usual strength. 1 per cent w/v.

Identification

In the Assay the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.5 to 8.5.

Impurity A. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the ophthalmic suspension containing 10 mg of brinzolamide to 25.0 ml with *ethanol*.

Reference solution. A solution containing 0.04 per cent w/v of *brinzolamide IPRS* and 0.002 per cent w/v solution of *brinzolamide impurity A IPRS (brinzolamide-(S)-isomer IPRS)* in *ethanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with amylase tris-3,5-dimethylphenylcarbamate coated to porous silica (5 µm) (Such as chiral Pack ADH),
- mobile phase: a mixture of 55 volumes of *ethanol*, 40 volumes of *hexane*, 5 volumes of *methanol* and 0.2 volume of *diethylamine*,
- flow rate: 0.75 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5 µl.

The relative retention time with reference to brinzolamide for brinzolamide impurity A is about 1.2.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to brinzolamide and brinzolamide impurity A is not less than 1.8, the column efficiency is not less than 2000 theoretical plates and the tailing factor for the brinzolamide peak is not more than 1.8.

Inject the test solution. The area of any peak due to brinzolamide impurity A is not more than 1.5 per cent, calculated by area normalisation method.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the ophthalmic suspension containing 10 mg of brinzolamide to 50.0 ml with the mobile phase.

Reference solution (a). A 0.02 per cent w/v solution of *brinzolamide IPRS* in the mobile phase.

Reference solution (b). A 0.006 per cent w/v solution of (*R*-4-Amino)-2,3-dihydro-2-(3-methoxypropyl)-4*H* -thieno[3,2,-e]-thiazine-6-sulphonamide-1,1-dioxide ethandioate 1:1 *(brinzolamide impurity B IPRS)* in reference solution (a).

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of buffer solution prepared by dissolving 11.75 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 5.2 with *acetic acid* and 35 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The relative retention time with reference to brinzolamide for brinzolamide impurity B is between 0.48 and 0.61.

Inject reference solution (b). The test is not valid unless the resolution between brinzolamide and brinzolamide impurity B is not less than 4.5, the column efficiency is not less than 2500 theoretical plates and the tailing factor is not more than 2.0 for principal peak.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of the areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization method.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Dilute a volume of ophthalmic suspension containing 10 mg of brinzolamide to 50.0 ml with the mobile phase.

Reference solution (a). A 0.02 per cent w/v solution of *brinzolamide IPRS* in the mobile phase.

Reference solution (b). A 0.006 per cent w/v solution of *brinzolamide impurity B IPRS* in reference solution (a).

Use chromatographic system as described under Related substances.

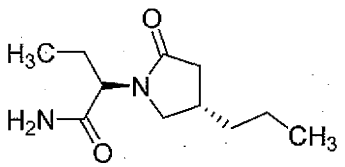
The relative retention time with reference to brinzolamide for brinzolamide impurity B is between 0.48 and 0.61.

Inject reference solution (a) and (b). The test is not valid unless the resolution between brinzolamide and brinzolamide impurity B is not less than 4.5, the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 for principal peak obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent for reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{12}H_{21}N_3O_5S_3$ in the suspension.

Brivaracetam



$C_{11}H_{20}N_2O_2$ Mol. Wt. 212.3

Brivaracetam is (2S)-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl]butanamide.

Brivaracetam contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{11}H_{20}N_2O_2$, calculated on the dried basis.

Category. Anticonvulsant.

Description. A white to off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *brivaracetam IPRS* or with the reference spectrum of brivaracetam.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). −65.0° to −55.0°, determined in 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (a). A 0.0003 per cent w/v solution of *brivaracetam IPRS* in mobile phase A.

Reference solution (b). Dilute 3.0 ml of reference solution (a) to 10.0 ml with mobile phase A.

Reference solution (c). A solution containing 0.0003 per cent w/v of *(2R,4R)-diastereomer* (*RR*-isomer of brivaracetam) *IPRS* or *(2S,4S)-diastereomer* (*SS*-isomer of brivaracetam) *IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane with extra selectivity of penta fluoro phenyl phase bonded to porous silica (5 µm) (Such as ACE C18-PFP),
- column temperature: 35°,
- sample temperature: 10°,
- mobile phase: A. a 0.1 per cent v/v solution of *ortho-phosphoric acid* in *water*,
  B. a mixture of 40 volumes mobile phase A, 30 volumes of *methanol* and 30 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 15 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
20	50	50
50	50	50
50.1	100	0
60	100	0

The relative retention time with reference to brivaracetam for *RR* isomer or *SS* isomer of brivaracetam is about 1.02.

Inject reference solution (a) and (b). The test is not valid unless the tailing factor is not more than 1.5, the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (a). The signal-to-noise ratio is not less than 10 in the chromatogram obtained with reference solution (b).

Inject reference solution (c) to identify the peak due to RR isomer or SS isomer of brivaracetam.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak corresponding to *RR* isomer and *SS* isomer of brivaracetam.

The sum of all the impurities including RR isomer of brivaracetam and SS isomer of brivaracetam (determined under chiral purity test) is not more than 1.0 per cent.

Chiral purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A 0.001 per cent w/v solution of *brivaracetam IPRS* in the mobile phase.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 20.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.006 per cent w/v, each of, *enantiomer of brivaracetam IPRS* and *(2R,4R)-diastereomer* (*RR* isomer of brivaracetam) *IPRS* and *(2S,4S)-diastereomer* (*SS* isomer of brivaracetam) *IPRS* in the mobile phase.

Reference solution (d). Dissolve 20 mg of *brivaracetam IPRS* in the mobile phase, add 1.0 ml of reference solution (c) and dilute to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with amylose tris-(3,5-dimethylphenylcarbamate) coated with porous silica (5 µm) (Such as chiralpack AD-H),
- column temperature: 15 °,
- sample temperature: 10°,
- mobile phase: a mixture of 85 volumes of *n-hexane* and 15 volumes of *ethanol*.
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Name	Relative retention time
Enantiomer of brivaracetam[1]	0.39
RR-isomer of brivaracetam[2]	0.43
SS-isomer of brivaracetam[3]	0.65
Brivaracetam (Retention time is about 36 minutes)	1.0

[1](2*R*)-2-[(4*S*)-2-oxo-4-propylpyrrolidin-1-yl]butanamide,

[2](2*R*)-2-[(4*R*)-2-oxo-4-propylpyrrolidin-1-yl]butanamide,

[3](2*S*)-2-[(4*S*)-2-oxo-4-propylpyrrolidin-1-yl]butanamide.

Inject reference solution (a), (b) and (d). The test is not valid unless the resolution between the peaks due to enantiomer of brivaracetam and (2*R*,4*R*)-diastereomer is not less than 1.8 in the chromatogram obtained with reference solution (d), the

tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (a). The signal-to-noise ratio of the principal peak is not less than 10 in the chromatogram obtained with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to enantiomer, *RR*-isomer and *SS*-isomer of brivaracetam, each of, is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g in vacuum oven at 60° for 3 hours, at a pressure not exceeding 0.7 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Solution A. A 0.1 per cent v/v solution of *orthophosphoric acid* in *water*.

Test solution. Dissolve 100 mg of the substance under examination in solution A and dilute to 100.0 ml with solution A. Dilute 2.0 ml of the solution to 10.0 ml with solution A.

Reference solution (a). A 0.02 per cent w/v solution of *brivaracetam IPRS* in solution A.

Reference solution (b). A solution containing 0.02 per cent w/v of *brivaracetam IPRS* and 0.00006 per cent w/v of *(2R,4R)-diastereomer* or *(2S ,4S)-diastereomer IPRS* in solution A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane with extra selectivity of a penta fluoro phenyl bonded to porous silica (5 µm) (Such as ACE-C18-PFP),
- mobile phase: a mixture of 64 volumes of solution A, 18 volumes of *acetronitrile* and 18 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 15 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to brivaracetam and (2*R*,4*R*)-diastereomer or (2*S*,4*S*)- diastereomer is not less than 1.8 in the chromatogram obtained with reference solution (b), the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{11}H_{20}N_2O_2$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Brivaracetam Tablets

Brivaracetam Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of brivaracetam, $C_{11}H_{20}N_2O_2$.

Usual strengths. 25 mg; 50 mg; 75 mg; 100 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 0.5 g of *sodium hydroxide* in 1000 ml of *water*, adjusted to pH 6.4 with *1 M sodium hydroxide*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and centrifuge at 4000 rpm for 10 minutes.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of 0.1 per cent v/v solution of *orthophosphoric acid* in *water*, 10 volumes of *methanol* and 10 volumes of *acetonitrile*.

Test solution. Use the clear supernatant liquid, dilute if necessary with the dissolution medium.

Reference solution. A 0.1 per cent w/v solution of *brivaracetam IPRS* in the solvent mixture. Diluted 3.0 ml of the solution to 100.0 ml with the dissolution medium.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as X Bridge C18),
- column temperature: 35°,
- mobile phase: a mixture of 80 volumes of 0.1 per cent v/v solution of *orthophosphoric acid* in *water* and 20 volumes of a mixture of 90 volumes of *acetonitrile* and 10 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{20}N_2O_2$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{11}H_{20}N_2O_2$.

Related substances. Determine by liquid chromatography (2.4.14), as described under Dissolution with the following modifications.

Test solution. Disperse sufficient quantity of intact tablets containing 0.3 g of Brivaracetam in the solvent mixture, with the aid of ultrasound with intermediate shaking and dilute to 200.0 ml with the solvent mixture. Centrifuge at 4000 rpm for 15 minutes. Dilute 4.0 ml of supernatant liquid to 25.0 ml with the solvent mixture.

Reference solution. A 0.024 per cent w/v solution of *brivaracetam IPRS* in the solvent mixture.

Inject the reference solution .The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of area of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization.

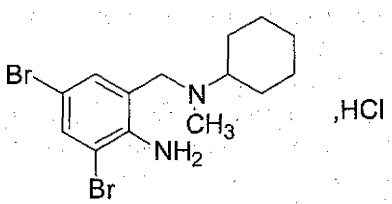
Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{20}N_2O_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Bromhexine Hydrochloride



$C_{14}H_{20}Br_2N_2,HCl$ Mol. Wt. 412.6

Bromhexine Hydrochloride is 2,4-Dibromo-6-[[cyclohexyl (methyl)amino]methyl]aniline hydrochloride.

Bromhexine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{20}Br_2N_2,HCl$, calculated on the dried basis.

Category. Expectorant.

Description. A white or almost white, crystalline powder. It shows polymorphism (2.5.11).

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bromhexine hydrochloride IPRS* or with the reference spectrum of bromhexine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (c).

C. Dissolve about 20 mg in 1 ml of *methanol* and add 1 ml of *water*. The solution gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 3.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14)

Solvent mixture. Equal volumes of *acetonitrile* and *water.*

Test solution (a). Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Test solution (b). Dilute 1.0 ml of test solution (a) to 50.0 ml with the solvent mixture.

Reference solution (a). Dissolve 10 mg of *bromhexine for system suitability IPRS* (containing impurities C and D) in the solvent mixture and dilute to 2.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (c). A 0.01 per cent w/v solution of *bromhexine hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 2.1 mm, packed with end-capped octadecylsilane bonded to porous silica (2.6 µm),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 1.26 g of *ammonium formate* in 950 ml of *water,* adjusted to pH 4.4 with *anhydrous formic acid,* dilute to 1000 ml with *water* and 40 volumes of *acetonitrile,*
- flow rate: 0.2 ml per minute,
- spectrophotometer set 248 nm,
- injection volume: 3 µl.

Name	Relative retention time
Bromhexine impurity C[1]	0.2
Bromhexine impurity D[2]	0.3
Bromhexine (Retention time: about 10 minutes)	1.0

[1]2-[[cyclohexyl(methyl)amino]methyl]aniline,

[2]4-bromo-2-[[cyclohexyl(methyl)amino]methyl]aniline.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to bromhexine impurity C and bromhexine impurity D is not less than 2.0.

Inject reference solution (b) and test solution (a). Run the chromatogram twice the retention time of the principal peak, the area of any peak corresponding to impurity C, multiplied with correction factor of 1.6, is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent)

Sulphated ash (2.3.18).Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Inject reference solution (c) and test solution (b).

Calculate the content of $C_{14}H_{20}Br_2N_2,HCl$.

Storage. Store protected from light.

Bromhexine Tablets

Bromhexine Hydrochloride Tablets

Bromhexine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of bromhexine hydrochloride, $C_{14}H_{20}Br_2N_2$, HCl.

Usual strengths. 4 mg; 8 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (c).

B. Suspend a quantity of the powdered tablets containing 0.1 g of Bromhexine Hydrochloride in 5 ml of *dilute ammonia solution* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined extracts with 5 ml of *water*, filter through anhydrous *sodium sulphate* and evaporate the filtrate to dryness using a rotary evaporator. If necessary, scratch the inside of the flask with a glass rod to induce crystallisation. Mix the residue with 1 g of *sodium carbonate*, heat at a dull red heat for 10 minutes, allow to cool, extract with water and filter. The filtrate, after acidification with *2 M nitric acid*, yields reaction A of bromides (2.3.1).

C. Disperse a quantity of the powdered tablets containing 20 mg of Bromhexine Hydrochloride with 10 ml *methanol* and filter. The filtrate gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14)

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution (a). Disperse a quantity of the powdered tablets containing 50 mg of Bromhexine Hydrochloride in 10 ml of solvent mixturewith the aid of ultrasound and filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 50.0 ml with the solvent mixture.

Reference solution (a). Dissolve 10 mg of *bromhexine for system suitability IPRS* (containing impurities C and D) in the solvent mixture and dilute to 2.0 ml with the solvent mixture.

Reference solution (b). A 0.0005 per cent w/v solution of *bromhexine hydrochloride IPRS* in the solvent mixture.

Reference solution (c). A 0.01 per cent w/v solution of *bromhexine hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 2.1 mm, packed with end-capped octadecylsilane bonded to porous silica (2.6 µm),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 1.26 g of *ammonium formate in* 950 ml of *water*, adjusted to pH 4.4 with *anhydrous formic acid*, dilute to 1000 ml with *water* and 40 volumes of *acetonitrile*,
- flow rate: 0.2 ml per minute,
- spectrophotometer set 248 nm,
- injection volume: 3 µl.

Name	Relative retention time
Bromhexine impurity C[1]	0.2
Bromhexine impurity D[2]	0.3
Bromhexine (Retention time: about 10 minutes)	1.0

[1]2-[[cyclohexyl(methyl)amino]methyl]aniline,

[4-bromo-2-[[cyclohexyl(methyl)amino]methyl]aniline.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to bromhexine impurity C and D is not less than 2.0.

Inject reference solution (b) and test solution (a). Run the chromatogram twice the retention time of the principal peak, the area of any peak corresponding to impurity C, multiplied with correction factor of 1.6, is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent)

Other tests. Comply with the tests stated under Tablets.

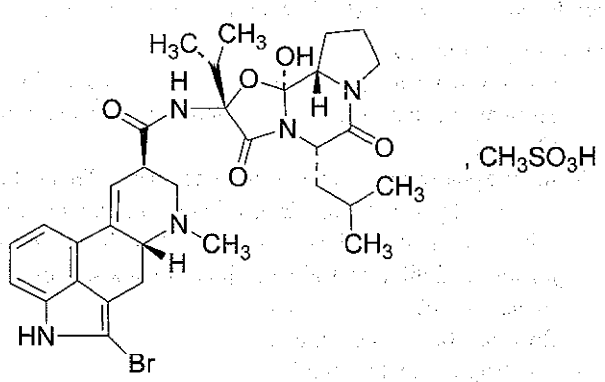
Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Inject reference solution (c) and test solution (b).

Calculate the content of $C_{14}H_{20}Br_2N_2,HCl$ in the tablets.

Storage. Store protected from light.

Bromocriptine Mesylate



$C_{32}H_{40}BrN_5O_5,CH_4O_3S$ Mol. Wt. 750.7

Bromocriptine Mesylate is (5'*S*)-2-bromo-12'-hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl)ergotaman-3',6',18-trione methanesulphonate

Bromocriptine Mesylate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{32}H_{40}BrN_5O_5,CH_4O_3S$, calculated on the dried basis.

Category. Antiparkinson.

Description. A white or slightly coloured, fine crystalline powder; very sensitive to light.

NOTE — Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry in a mineral oil dispersion (2.4.6). Compare the spectrum with that obtained with *bromocriptine mesylate IPRS* or with the reference spectrum of bromocriptine mesylate.

B. Dissolve 5 mg in 5 ml of *methanol* and dilute to 100 ml with *0.01 M hydrochloric acid*. The resulting solution, when examined in the range 230 nm to 360 nm (2.4.7) shows an absorption maximum at about 305 nm and a minimum at about 270 nm; absorbance at about 305 nm, 0.60 to 0.68.

C. To about 0.1 g add 5 ml of *2 M hydrochloric acid*, shake for 5 minutes, filter and add 1 ml of a 6 per cent w/v solution of *barium chloride* to the filtrate; it remains clear. Mix another 0.1 g with 0.5 g of *anhydrous sodium carbonate* and ignite until a white residue is obtained. After cooling, dissolve the residue in 5 ml of *water* (solution A); solution A gives the reactions of sulphates (2.3.1).

D. Solution A gives reaction (A) of bromides (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution BS5, YS5 or BYS5 (2.4.1).

pH (2.4.24). 3.1 to 3.8, determined in a 1.0 per cent w/v solution in a mixture of 2 volumes of *methanol* and 8 volumes of *water*.

Specific optical rotation (2.4.22). +95° to +105°, determined in a 1.0 per cent w/v solution in a mixture of equal volumes of *methanol* and *dichloromethane*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 50 volumes of *chloride buffer pH 2.0* and 50 volumes of *methanol*.

Test solution. Dissolve 0.5 g of the substance under examination in 5.0 ml of *methanol* and dilute to 10.0 ml with *chloride buffer pH 2.0*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c). Dissolve the contents of a vial of *bromocriptine mesylate for system suitability IPRS* (containing bromocriptine impurities A and B) in 1.0 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 12 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: A. a 0.079 per cent w/v solution of *ammonium carbonate,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
30	40	60
45	40	60
47	90	10

Use the chromatogram supplied with bromocriptine mesylate for system suitability RS and the chromatogram obtained with reference solution (c) to identify the peaks due to bromocriptine impurities A and B. The relative retention time with reference to bromocriptine for bromocriptine impurity C is about 1.2.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to 2-bromodehydro-α-ergocriptine (bromocriptine impurity A) and α-ergocriptine (bromocriptine impurity B) is not less than 1.1.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak corresponding to bromocriptine impurity A is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent), the area of secondary peak corresponding to bromocriptine impurity C is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent), the area of any other secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) (except the peak due to bromocriptine impurity A).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 0.5 g by drying in an oven over *phosphorus pentoxide* at 80° at a pressure of 1.5 to 2.5 kPa for 5 hours.

Assay. Weigh 0.5 g, dissolve in 80 ml of a mixture of 10 volumes of *anhydrous glacial acetic acid* and 70 volumes of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25) Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.07507 g of $C_{32}H_{40}BrN_5O_5,CH_4O_3S$.

Storage. Store protected from light in a deep freezer (temperature not exceeding –15°).

Bromocriptine Capsules

Bromocriptine Mesylate Capsules

Bromocriptine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bromocriptine, $C_{32}H_{40}BrN_5O_5$.

Usual strengths. 5 mg; 10 mg.

NOTE — Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.

Identification

A. Disperse a quantity of the contents of the capsules containing 10 mg of bromocriptine with 50 ml of *methanol* for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 20 ml with *methanol*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 305 nm and a minimum at about 270 nm.

B. In the test for Related substances, the principal band in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with reference solution (e).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium.500 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a suitable quantity of *bromocriptine mesylate IPRS* in minimum amount of *methanol* and dilute with the dissolution medium to obtain a solution having the same concentration as that of the test solution.

Chromatographic system
- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *acetonitrile* and 35 volumes of *0.01M ammonium carbonate*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{32}H_{40}BrN_5O_5$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{32}H_{40}BrN_5O_5$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

NOTE — Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.

Mobile phase. A mixture of 0.1 volume of *13.5M ammonia*, 1.5 volumes of *water*, 3 volumes of *propan-2-ol*, 88 volumes of *dichloromethane* and 100 volumes of *ether.*

Test solution. Disperse a quantity of the contents of the capsules containing about 20 mg of bromocriptine with 10 ml of *methanol* for 20 minutes and centrifuge.

Reference solution (a). Dilute 1 ml of the test solution to 10 ml with *methanol.*

Reference solution (b). Dilute 3 ml of the test solution to 100 ml with *methanol.*

Reference solution (c). Dilute 1 ml of the test solution to 100 ml with *methanol*

Reference solution (d). Dilute 1 ml of the test solution to 200 ml with *methanol.*

Reference solution (e). A 0.23 per cent w/v solution of *bromocriptine mesilate IPRS* in *methanol.*

Apply to the plate 50 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of cold air, spray with *ammonium molybdate solution* and heat at 100° until bands appear (about 10 minutes). Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) (3.0 per cent), not more than one such secondary spot is more intense than the chromatogram obtained with reference solution (c) (1.0 per cent) and not more than two such secondary spots are more intense than the chromatogram obtained with reference solution (d) (0.5 per cent). Ignore the spot within 20 mm of the line of application.

Uniformity of content. Complies with the test stated under Capsules.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse the content of one capsule in the solvent mixture and dilute with the solvent mixture to obtain a solution containing 0.011 per cent w/v of Bromocriptine.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{32}H_{40}BrN_5O_5$ in the capsule.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water.*

NOTE — Prepare the solutions under subdued light.

Test solution. Weigh a quantity of the mixed content of 20 capsules containing 10 mg of bromocriptine, disperse in 70 ml of the solvent mixture, with the aid of ultrasound for 5 minutes and dilute to 100.0 ml with the solvent mixture and filter.

Reference solution (a). A 0.011 per cent w/v solution of *bromocriptine mesylate IPRS* in the solvent mixture.

Reference solution (b). A 0.011 per cent w/v solution of *bromocriptine mesylate IPRS* in a mixture of 1 volume of *1M acetic acid* and 9 volumes of *methanol*, heat at 60° for 90 minutes and cool to room temperature.

Chromatographic system
- a stainless steel column 10 cm x 4 mm, packed with octadecylsilane bonded to silica (5µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 45 volumes of a 0.08 per cent w/v solution of *ammonium carbonate* and 55 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two peaks is not less than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{32}H_{40}BrN_5O_5$ in the capsules.

1 mg of bromocriptine mesylate, $C_{32}H_{40}BrH_5O_5,CH_4SO_3$ is equivalent to 0.87 mg of bromocriptine, $C_{32}H_{40}BrH_5O_5$

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of bromocriptine.

Bromocriptine Tablets

Bromocriptine Mesylate Tablets

Bromocriptine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of bromocriptine, $C_{32}H_{40}BrN_5O_5$.

Usual strengths. 1 mg; 2.5 mg.

NOTE — Carry out the tests as rapidly as possible without exposure to daylight and with minimum exposure to artificial light.

Identification

A. Disperse a quantity of powdered tablets containing about 20 mg of bromocriptine with 20 ml of *methanol*, filter, evaporate the filtrate to dryness on a water-bath and dry at 105° for 1 hour.The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bromocriptine mesylate IPRS* or with the reference spectrum of bromocriptine mesylate.

B. Disperse a quantity of the powdered tablets containing about 10 mg of bromocriptine with 50 ml of *methanol* for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 20 ml with *methanol*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 305 nm and a minimum at about 270 nm.

C. In the test for Related substances, the principal band in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with reference solution (e).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a suitable quantity of *bromocriptine mesylate IPRS* in minimum amount of *methanol* and dilute with the dissolution medium to obtain a solution having the same concentration as that of the test solution.

Chromatographic system
- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *acetonitrile* and 35 volumes of *0.01M ammonium carbonate*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{32}H_{40}BrN_5O_5$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{32}H_{40}BrN_5O_5$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Solvent mixture. Equal volumes of *chloroform* and *methanol.*

Mobile phase. A mixture of 0.1 volume of *13.5M ammonia*, 1.5 volumes of *water*, 3 volumes of *propan-2-ol*, 88 volumes of *dichloromethane* and 100 volumes of *ether.*

Test solution. Disperse a quantity of the powdered tablets containing about 10 mg of bromocriptine with 25 ml of the solvent mixture for 30 minutes, filter and wash the residue with two 5 ml quantities of the solvent mixture. Evaporate the filtrate and washings to dryness at 25° at a pressure of 2 kPa, dissolve the residue in 2 ml of the solvent mixture and centrifuge.

Reference solution (a). Dilute 1 ml of the test solution to 10 ml with the solvent mixture.

Reference solution (b). Dilute 3 ml of reference solution (a) to 10 ml with the solvent mixture

Reference solution (c). Dilute 1 ml of reference solution (a) to 10 ml with the solvent mixture.

Reference solution (d). Dilute 1 ml of reference solution (a) to 20 ml with the solvent mixture.

Reference solution (e). A 0.55 per cent w/v solution of *bromocriptine mesilate IPRS* in the solvent mixture.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air, spray with *ammonium molybdate solution* and heat at 100° until spots appear (about 10 minutes). Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) (3.0 per cent), not more than one such secondary spot is more intense than the spot in the chromatogram obtained with reference solution (c) (1.0 per cent) and not more than two secondary

spots are more intense than the spot in the chromatogram obtained with reference solution (d) (0.5 per cent). Ignore the spot within 20 mm of the line of application.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in the solvent mixture and dilute with the solvent mixture to obtain a solution containing 0.011 per cent w/v of Bromocriptine.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{32}H_{40}BrN_5O_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water*.

NOTE — Preparethe solutions under subdued light.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 10 mg of bromocriptine, disperse in 70 ml of the solvent mixture, with the aid of ultrasound for 5 minutes and dilute to 100.0 ml with the solvent mixture and filter.

Reference solution (a). A 0.011 per cent w/v solution of *bromocriptine mesylate IPRS* in the solvent mixture.

Reference solution (b). A 0.011 per cent w/v solution of *bromocriptine mesylate IPRS* in a mixture of 1 volume of *1M acetic acid* and 9 volumes of *methanol*, heat at 60° for 90 minutes and cool to room temperature.

Chromatographic system

- a stainless steel column 10 cm x 4 mm, packed with octadecylsilane bonded to silica (5µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 45 volumes of a 0.08 per cent w/v solution of *ammonium carbonate* and 55 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two peaks is not less than 3.0.

Inject reference solution (a) and the test solution.

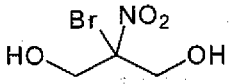
Calculate the content of $C_{32}H_{40}BrN_5O_5$ in the tablets.

1 mg of bromocriptine mesylate, $C_{32}H_{40}BrH_5O_5,CH_4SO_3$ is equivalent to 0.87 mg of bromocriptine, $C_{32}H_{40}BrH_5O_5$

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of bromocriptine.

Bronopol



$C_3H_6BrNO_4$ Mol. Wt. 200.0

Bronopol is 2-bromo-2-nitropropane-1,3-diol.

Bronopol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_3H_6BrNO_4$, calculated on the anhydrous basis.

Category. Antiseptic; local anaesthetic.

Description. White or almost white crystals or crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bronopol IPRS* or with the reference spectrum of bronopol.

B. Dissolve 0.1 g in 10 ml of *water*, add 10 ml of *7.5 M sodium hydroxide* and carefully with constant stirring and cooling, 0.5 g of *nickel-aluminium alloy*. Allow the reaction to subside, filter and carefully neutralise with *nitric acid*. The resulting solution gives reaction (A) of bromides (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.0, determined on 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of mobile phase.

Reference solution (a). Dilute 5 ml of the test solution to 50 ml with the mobile phase. Further, dilute 1 ml of the solution to 100 ml with the mobile phase.

Reference solution (b). A solution containing 0.001 per cent w/v each of *2-methyl-2-nitropropan-1,3-diol* and *tris (hydroxymethyl)nitromethane* in the mobile phase.

Reference solution (c). A solution containing 0.0002 per cent w/v each of *2-methyl-2-nitropropane-1,3-diol, 2-nitroethanol, sodium bromide* and *tris(hydroxymethyl)-nitromethan* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 189 volumes of *water*, 10 volumes of *acetonitrile* and 1 volume of a 10 per cent v/v solution of *orthophosphoric acid*, adjusted to pH 3.0 using *2 M sodium hydroxide*,
- flow rate: 1 ml per minute,

- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to sodium bromide and tris (hydroxymethyl) nitromethane is at least 1.0 and the resolution between the peaks corresponding to tris(hydroxymethyl)nitromethane and 2-nitroethanol is at least 1.5.

Inject reference solution (a), (b) the test solution. Continue the chromatography for 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peaks corresponding to 2-methyl-2-nitropropane-1,3-diol and tris (hydroxymethyl) nitromethane are not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (b) (0.5 per cent each) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 2.0 g.

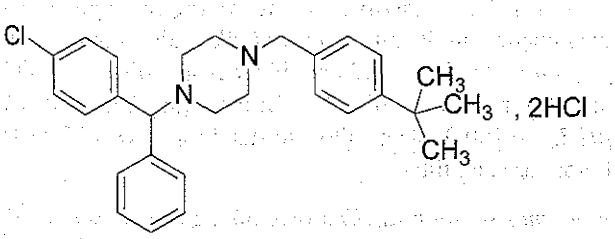
Assay. In a flask fitted with a reflux condenser dissolve 0.4 g in 15 ml of *water* and add 15 ml of *7.5 M sodium hydroxide*. Slowly, with caution, add 2 g of *nickel-aluminium alloy* through the reflux condenser, agitating the flask whilst cooling under running *water*. Allow the mixture to stand for 10 minutes and boil for 1 hour. Cool and filter under reduced pressure, washing the condenser, flask and residue with 150 ml of *water*. Combine the filtrate and washings, add 25 ml of *nitric acid* and 40 ml of *0.1 M silver nitrate*, shake vigorously and titrate with *0.1 M ammonium thiocyanate* using *ammonium iron(III) sulphate solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.020 g of $C_3H_6BrNO_4$.

Storage. Store protected from light.

Buclizine Hydrochloride

Buclizine Dihydrochloride



$C_{28}H_{33}ClN_2,2HCl$ Mol. Wt. 506.0

Buclizine Hydrochloride is (*RS*)-1-(4-*tert*-butylbenzyl)-4-(4-chlorobenzhydryl)piperazine dihydrochloride

Buclizine Hydrochloride contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{28}H_{33}ClN_2,2HCl$, calculated on the dried basis.

Category. Antihistaminic; antiemetic.

Description. A white or slightly yellowish, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *buclizine dihydrochloride IPRS* or with the reference spectrum of buclizine dihydrochloride.

B. A 0.25 per cent w/v solution in *ethanol (50 per cent)* gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 0.5 g of the substance under examination in 100 ml of the initial mobile phase.

Test solution (b). Dissolve 10 mg of the substance under examination in 100 ml of the initial mobile phase and mix. Dilute 10 ml of the solution to 100 ml with the same mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *buclizine dihydrochloride IPRS* in the initial mobile phase.

Reference solution (b). A 0.001 per cent w/v solution of *buclizine impurity A IPRS* (*1,4-bis(4-chlorobenzylhydryl) piperazine IPRS*) in the initial mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Nucleosil C 18),
- mobile phase, initial: *0.01 M sodium heptanesulphonate* in a mixture of 55 volumes of *water* and 45 volumes of *acetonitrile*, adjusted to pH 4.0 with *1 M orthophosphoric acid*, final: *0.01 M sodium heptanesulphonate* in a mixture of 20 volumes of *water* and 80 volumes of *acetonitrile*, adjusted to pH 4.0 with *1 M ortho-phosphoric acid*, a linear gradient elution programme for 30 minutes with the initial mobile phase and 10 minutes with the final mobile phase,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation is not more than 2.0 per cent.

Inject reference solution (b), test solution (a) and (b). In the chromatogram obtained with test solution(a), the area of any peak corresponding to impurity A is not more than the area of

the peak obtained in the chromatogram obtained with reference solution (b) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with test solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

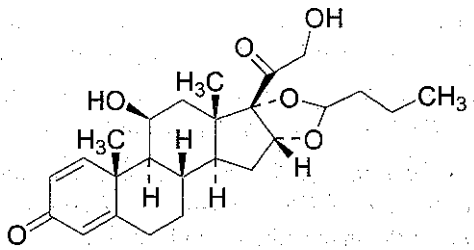
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.4 g in 50 ml of *anhydrous acetic acid*, add 10 ml of mercuric acetate solution. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0253 g of $C_{28}H_{33}ClN_2,2HCl$.

Storage. Store protected from light and moisture.

Budesonide



$C_{25}H_{34}O_6$ Mol. Wt. 430.5

Budesonide is a mixture of the C-22*S* (epimer A) and the C-22*R* (epimer B) epimers of 16α,17-[(1*RS*)-butylidenebis(oxy)]-11β,21-dihydroxypregna-1,4-diene-3,20-dione.

Budesonide contains not less than 98.0 per cent and not more than 102.0 per cent of a mixture of epimers A and B, $C_{25}H_{34}O_6$, calculated on the dried basis.

Category. Glucocorticoid.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *budesonide IPRS* or with the reference spectrum of budesonide.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Add a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

Solvent mixture. 1 volume of *methanol* and 9 volumes of *methylene chloride*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). A 0.25 per cent w/v solution of *budesonide IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.25 per cent w/v of *triamcinolone acetonide IPRS* and 0.25 per cent w/v of *budesonide IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve about 2 mg in 2 ml of *sulphuric acid*. A yellow colour appears in 5 minutes and the colour changes to brown or reddish-brown in 30 minutes. Add cautiously the solution to 10 ml of *water* and mix. The colour fades and a clear solution remains.

D. Dissolve about 1 mg in 2 ml of a solution containing 2 g of *phosphomolybdic acid* in a mixture of 10 ml of *dilute sodium hydroxide solution*, 15 ml of *water* and 25 ml of *glacial acetic acid*. Heat for 5 minutes on a water-bath. Cool in iced water for 10 minutes and add 3 ml of *dilute sodium hydroxide solution*. The solution turns blue.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 30 ml of *acetonitrile*. Add about 60 ml of *phosphate buffer pH 3.2* and, if necessary, disperse with the aid of ultrasound to dissolve. Dilute with *phosphate buffer pH 3.2* to 100.0 ml and allow to stand for at least 15 minutes before use and filter.

Reference solution (a). Dissolve 50 mg of *budesonide IPRS* in 30 ml of *acetonitrile*. Add about 60 ml of *phosphate buffer pH 3.2* and disperse, if necessary, with the aid of ultrasound to dissolve. Dilute to 100.0 ml *with phosphate buffer pH 3.2* and allow to stand for at least 15 minutes before use and filter.

Reference solution (b). Dilute reference solution (a) with the mobile phase to get a 0.00025 per cent w/v solution of budesonide.

Use the chromatographic system described under Assay.

Inject reference solution (a). The test is not valid unless the resolution between epimer B peak and epimer A peak is not less than 1.5, the tailing factor for epimer B peak is not more than 1.5 and the relative standard deviation of sum of epimer A and epimer B peaks for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution: the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the peaks, other than the principal peak, is not greater than thrice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 30 ml of *acetonitrile* and dilute to 100.0 ml with *phosphate buffer solution pH 3.2* and filter.

Reference solution. Dissolve 50 mg of *budesonide IPRS* in 30 ml of *acetonitrile* and dilute to 100.0 ml with *phosphate buffer solution pH 3.2*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 34 volumes of *acetonitrile* and 66 volumes of a buffer solution prepared by adding 100 ml of 0.25 per cent w/v solution of *orthophosphoric acid* to 900 ml of 0.4 per cent w/v solution of *sodium dihydrogen phosphate* and adjusted to pH 3.2,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between epimer B peak and epimer A peak is not less than 1.5, the tailing factor for epimer B peak is not more than 1.5, the column efficiency determined for epimer B peak is not less than 4000 theoretical plates and the relative standard deviation for the sum of epimer A and B peaks for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{34}O_6$.

Storage. Store protected from light.

Budesonide Inhalation

Budesonide Inhalation is a suspension of Budesonide in a suitable liquid, filled in a suitable pressurized container.

Budesonide Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of budesonide, $C_{25}H_{34}O_6$ per inhalation by actuation of the valve.

Usual strengths. 100 µg; 200 µg.

Identification

A. Dilute a quantity of the inhalation with *water* to produce a solution containing 0.002 per cent w/v of Budesonide and filter. When examined in the range 200 nm to 350 nm (2.4.7), the solution exhibits a maximum only at about 247 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Solvent mixture. 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer pH 3.2*.

Test solution. Discharge the container into a small, dry flask a sufficient number of times to obtain 1 mg of Budesonide and dissolve the residue in 3.4 ml of *acetonitrile*. Mix with the aid of ultrasound and add sufficient *phosphate buffer solution pH 3.2* to produce 10 ml and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm) (Such as Spherisorb ODS-2),
- column temperature: 50°,
- mobile phase: A. a mixture of 2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer pH 3.2*,
  B. a mixture of equal volumes of *acetonitrile* and *phosphate buffer pH 3.2*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
38	100	0
50	0	100
60	0	100
61	100	0
70	100	0

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to budesonide epimer B and epimer A is not less than 1.5 in the chromatogram obtained with reference solution (a) and the signal to noise ratio of the peaks due to budesonide epimer A and epimer B is not less than 10.0 in the chromatogram obtained with reference solution (b).

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the sum of areas of epimer peaks in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of the areas of all the secondary peaks is not more than 3 times the sum of areas of epimer peaks in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than the sum of the areas of epimer peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

Epimer A. The content of epimer A (second peak) is 40.0 per cent to 51.0 per cent of the sum of areas of two epimer peaks of budesonide.

Determine by liquid chromatography (2.4.14), as described under Assay using the test solution.

Other tests. Comply with the tests stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Solvent mixture. 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer pH 3.2.*

Test solution. Determine the content of active ingredient by first 10 successive combined actuations of the valve after priming. Carry out the test for Content of active ingredient delivered by actuation of valve stated under Inhalation Preparations (Pressurised Metered-dose Preparations), beginning at the words 'Remove the pressurised container from the actuator ...' and ending at the words '... to the volume specified in the monograph', using 32 ml of *acetonitrile* in the vessel. Transfer the combined solution and washings obtained from the set of 10 combined actuations to a flask to obtain a solution containing 0.002 per cent w/v of Budesonide in the solvent mixture.

Reference solution. A 0.002 per cent w/v solution of *budesonide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm) (Such as Spherisorb ODS-2),
- column temperature: 50°,
- mobile phase: a mixture of 2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer pH 3.2*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to budesonide epimer B and epimer A is not less than 1.5.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{34}O_6$ in the inhalation from the sum of the areas of two budesonide epimer peaks. Determine the content of active ingredient a second and third time by repeating the procedure on the middle 10 and on the last 10 successive combined actuations of the valve, as estimated from the number of deliveries available from the container as stated on the label. For each of the three determinations the average content of $C_{25}H_{34}O_6$ delivered by a single actuation of the valve is within the limits stated under Content of budesonide.

Budesonide Powder for Inhalation

Budesonide Powder for Inhalation consists of Budesonide in microfine powder either alone or admixed with Lactose in a pre-metered unit for use in a suitable powder inhaler.

Budesonide Powder for Inhalation contains not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of budesonide, $C_{25}H_{34}O_6$ per unit dose.

Usual strengths. 100 µg; 200 µg.

Identification

A. Dilute a quantity of the powder for inhalation with sufficient *water* to produce a solution containing 0.002 per cent w/v of Budesonide and filter. When examined in the range 200 nm to 350 nm (2.4.7), the solution exhibits a maximum only at 247 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. *For products containing lactose*- disperse 0.25 g of the powder for inhalation in 5 ml of *water*. Add 5 ml of *6 M ammonia* and heat in a water-bath at 80° for 10 minutes; an orange-red colour is produced.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Solvent mixture. 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer pH 3.2.*

Test solution. Dissolve a quantity of the powder for inhalation containing 1 mg of Budesonide in 3.4 ml of *acetonitrile* with the aid of ultrasound and dilute to 10.0 ml with *phosphate buffer pH 3.2,* filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm) (Such as Spherisorb ODS-2),
- column temperature: 50°,
- mobile phase: A. a mixture of 2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer pH 3.2*,
  B. a mixture of equal volumes of *acetonitrile* and *phosphate buffer pH 3.2*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
38	100	0
50	0	100
60	0	100
61	100	0
70	100	0

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to budesonide epimer B and epimer A is not less than 1.5 in the chromatogram obtained with reference solution (a) and in the chromatogram obtained with reference solution (b), the signal to noise ratio of the peaks due to budesonide epimer A and epimer B is not less than 10.0.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the sum of areas of epimer peaks in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of the areas of all the secondary peaks is not more than 3 times the sum of areas of epimer peaks in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than the sum of the areas of the epimer peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

Epimer A. The content of epimer A (second peak) is 40.0 per cent to 51.0 per cent of the sum of areas of two epimer peaks of budesonide.

Determine by liquid chromatography (2.4.14), as described under Assay using reference solution (b).

Uniformity of delivered dose. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Solvent mixture. 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer pH 3.2.*

Test solution. Collect single doses of the powder for inhalation using the procedure described in Inhalation Preparations under (Powders for Inhalation) – Uniformity of delivered dose and dissolve the collected dose in sufficient of the solvent mixture to produce a solution containing 0.0004 per cent w/v of Budesonide.

Reference solution. A 0.001 per cent w/v solution of *budesonide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm) (Such as Spherisorb ODS-2),
- column temperature: 50°,
- mobile phase: a mixture of 2 volumes of *ethanol*, 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer pH 3.2*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to budesonide epimer B and epimer A is not less than 1.5.

Inject the reference solution and the test solution.

Calculate the content of budesonide, $C_{25}H_{34}O_6$ per delivered dose.

Other tests. Comply with the tests stated under Inhalation Preparations (Powders for Inhalation).

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Solvent mixture. 34 volumes of *acetonitrile* and 66 volumes of *phosphate buffer pH 3.2.*

Test solution. Dissolve a quantity of the mixed contents of capsules in sufficient of the solvent mixture to get a solution containing 0.01 per cent w/v of budesonide.

Reference solution (a). A 0.01 per cent w/v solution of *budesonide IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with the solvent mixture.

Use chromatographic system as described in Uniformity of delivered dose.

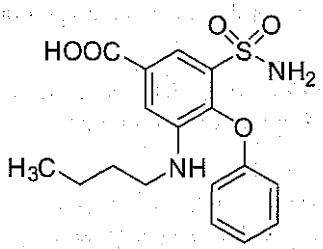
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to budesonide epimer B and epimer A is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{25}H_{34}O_6$ per unit dose.

Calculate the content of $C_{25}H_{34}O_6$ in the inhalation from the sum of the areas of two budesonide epimer peaks.

Bumetanide



$C_{17}H_{20}N_2O_5S$ Mol. Wt. 364.4

Bumetanide is 3-(aminosulphonyl)-5-(butylamino)-4-phenoxybenzoic acid

Bumetanide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{20}N_2O_5S$, calculated on the dried basis.

Category. Diuretic.

Description. A white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bumetanide IPRS* or with the reference spectrum of bumetanide.

Tests

Appearance of solution. A 0.5 per cent w/v solution in 0.6 per cent w/v solution of *potassium hydroxide* is clear (2.4.1) and colourless (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 25.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 2 mg each of *3-nitro-4-phenoxy-5-sulphamoylbenzoic acid (bumetanide impurity A IPRS)* and *3-amino-4-phenoxy-5-sulphamoylbenzoic acid (bumetanide impurity B IPRS)* in 10.0 ml of the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with encapped octylsilane bonded to porous silica (3.5 µm),
- mobile phase: a mixture of 70 volumes of *methanol,* 25 volumes of *water* and 5 volumes of a 2.72 per cent w/v solution of *potassium dihydrogen phosphate,* previously adjusted to pH 7.0 with 28 per cent w/v solution of *potassium hydroxide.* Add *tetrahexylammonium bromide* to this mixture to obtain a 0.22 per cent w/v solution,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to bumetanide impurity A and bumetanide impurity B is not less than 2.0. The relative retention time with reference to bumetanide for bumetanide impurity B is about 0.4, for bumetanide impurity A is about 0.6, for 3-[[(2*RS*)-2-ethylhexyl]amino]-4-phenoxy-5-sulphamoyl benzoic acid (bumetanide impurity D) is about 2.5 and for butyl 3-(butylamino)-4-phenoxy-5-sulphamoylbenzoate (bumetanide impurity C) is about 4.4.

Inject reference solution (a) and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of secondary peak corresponding to bumetanide impurities A, B, C and D is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with

reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh 0.3 g and dissolve in 50 ml of *ethanol (95 per cent)*. Add 0.1 ml of *phenol red solution* and titrate with *0.1 M sodium hydroxide* until a violet-red colour is obtained. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03644 g of $C_{17}H_{20}N_2O_5S$.

Storage. Store protected from light.

Bumetanide Injection

Bumetanide Injection is a sterile solution of Bumetanide in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Bumetanide Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bumetanide, $C_{17}H_{20}N_2O_5S$.

Usual strength. 0.25 mg per ml.

Identification

A. Disperse a quantity of the injection containing 10 mg of Bumetanide with 20 ml of *ether*, filter the ether layer through *anhydrous sodium sulphate* and evaporate. The residue complies with the following tests. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bumetanide IPRS* or with the reference spectrum of bumetanide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 6.0 to 7.8.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform*.

Test solution (a). To a quantity of the injection containing 5.0 mg of Bumetanide, adjusted to pH 12 with *0.1 M sodium hydroxide* and extract with two 20 ml quantities of *ether*. Discard the ether, adjusted to pH 4 using *1 M acetic acid*, extract with two further 20 ml quantities of *ether*, dry the ether by filtering through *anhydrous sodium sulphate*, wash the filter with 5 ml of *ether* and evaporate the combined filtrate and washings to dryness. Dissolve the residue in 5 ml of *methanol* and centrifuge. Evaporate the supernatant liquid to dryness using a rotary evaporator and dissolve the residue in 0.5 ml of *methanol*.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *methanol*. Dilute 1 ml of the solution to 30 ml with *methanol*.

Test solution (c). Dilute 1 ml of test solution (b) to 3 ml with *methanol*.

Reference solution. A 0.005 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid IPRS (bumetanide impurity A IPRS)* in *methanol*.

Apply to the plate 25 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) corresponding to 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent), any other secondary spot is not more intense than the spot in the chromatogram obtained with test solution (b) (0.3 per cent) and not more than two other such spots are more intense than the spot in the chromatogram obtained with test solution (c) (0.1 per cent).

Bacterial endotoxins (2.2.3). Not more than 350.0 Endotoxin Units per mg of bumetanide.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*.

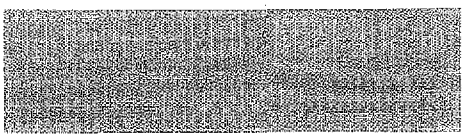
Test solution. Dilute a quantity of the injection containing 2.5 mg of Bumetanide to 25.0 ml with the solvent mixture.

Reference solution (a). A 0.025 per cent w/v solution of *bumetanide IPRS* in the solvent mixture. Dilute 10.0 ml of the solution to 25.0 ml with *water*.

Reference solution (b). A 0.0125 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid IPRS* in reference solution (a).

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as Bondapak ODS),



- mobile phase: a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to bumetanide and 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not less than 15.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the injection.

Bumetanide Oral Solution

Bumetanide Oral Solution is a solution of Bumetanide in a suitable flavoured vehicle.

The oral solution complies with the requirements stated under Oral Liquids and with the following requirements.

Bumetanide Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bumetanide, $C_{17}H_{20}N_2O_5S$.

Usual strength. 0.25 mg per ml.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform*.

Test solution (a). Mix a quantity of the oral solution containing 2 mg of Bumetanide with 10 ml of *water* and 0.6 ml of *1 M hydrochloric acid*, add 5 ml of *ethyl acetate*, shake for 15 minutes, centrifuge and decant the *ethyl acetate*. Add a further 5 ml of *ethyl acetate* to the residue, shake for 15 minutes, centrifuge and decant the *ethyl acetate*. Evaporate the combined ethyl acetate extracts to dryness and dissolve the residue in 0.5 ml of *methanol*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

Test solution (c). Dilute 1.0 ml of test solution (b) to 10.0 ml with *methanol*. Dilute 1 ml of the solution to 3 ml with *methanol*.

Test solution (d). Dilute 1.0 ml of test solution (c) to 100.0 ml with *methanol*.

Reference solution (a). A 0.04 per cent w/v solution of *bumetanide IPRS* in *methanol*.

Reference solution (b). A 0.002 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid IPRS* in *methanol*.

Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) corresponding to 3-amino-4-phenoxy-5-sulphamoyl-benzoic acid is not more intense than the spot in the chromatogram obtained with test solution (c) (0.5 per cent), any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.3 per cent) and not more than two other such spots are more intense than the spot in the chromatogram obtained with test solution (d) (0.1 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol*.

Test solution. Mix a quantity of the oral solution containing 2.5 mg of Bumetanide with 12.5 ml of *water* and 0.8 ml of *1 M hydrochloric acid*, add 10 ml of *ethyl acetate*, shake for 15 minutes, centrifuge and decant the *ethyl acetate*. Repeat the extraction procedure twice using a further two 10 ml quantities of *ethyl acetate* and beginning at the words 'add 10 ml of ... '. Evaporate the combined ethyl acetate extracts to dryness, dissolve the residue in 10 ml of the solvent mixture and dilute to 20 ml with *water*.

Reference solution (a). A 0.025 per cent w/v solution of *bumetanide IPRS* in the solvent mixture. Dilute 5 ml of the solution to 10.0 ml with *water*.

Reference solution (b). A 0.0125 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoyl-benzoic acid IPRS* in reference solution (a).

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as µBondapak ODS),
- mobile phase: a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to bumetanide and 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not less than 15.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in oral solution.

Determine the weight per ml of the oral solution (2.4.29) and calculate the content of $C_{17}H_{20}N_2O_5S$, weight in volume.

Bumetanide Tablets

Bumetanide Tablets contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of bumetanide, $C_{17}H_{20}N_2O_5S$.

Usual strengths. 0.5 mg; 1 mg; 2 mg.

Identification

A. Disperse a quantity of the powdered tablets containing 50 mg of Bumetanide with 25 ml of *ether*, filter through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bumetanide IPRS* or with the reference spectrum of bumetanide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.0056 per cent w/v solution of *bumetanide IPRS* in the solvent mixture. Dilute a suitable volume of the solution with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 70 volumes of buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.0 with *1.8 M potassium hydroxide* and 30 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{20}N_2O_5S$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform*.

Test solution. weigh and transfer a quantity of the powdered tablets containing 0.0125 g of bumetanide in 20 ml of a mixture of equal volumes of *acetonitrile* and *methanol*, shake for 20 minutes. Centrifuge for 10 minutes, decant and reserve the supernatant liquid. Extract the residue with 5 ml of a mixture of equal volumes of *acetonitrile* and *methanol*, shaking mechanically for 30 seconds, centrifuge for 10 minutes, decant and combine the extracts. Evaporate the combined extracts to dryness under reduced pressure, dissolve the residue in 0.5 ml of *methanol* and centrifuge for 10 minutes.

Reference solution (a). Dilute 0.3 ml of the test solution to 100 ml with *methanol*.

Reference solution (b). Dilute 0.1 ml of the test solution to 100 ml with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.3 per cent) and not more than three such spots are more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Disperse 1 tablet in 10.0 ml of the solvent mixture, shake with the aid of ultrasound for 5 minutes, dilute to 20.0 ml with *water* and filter.

Reference solution. A 0.01 per cent w/v solution of *bumetanide IPRS* in the solvent mixture. Dilute 10.0 ml of the solution to 20.0 ml with *water*.

Use the chromatographic system as described under Assay.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 2 volumes of *glacial acetic acid,* 5 volumes of *tetrahydrofuran* and 45 volumes of *methanol.*

Test solution. Weigh and powder 20 tablets. Disperse an quantity of the powder containing 2.5 mg of Bumetanide in 10 ml of the solvent mixture, shake for 5 minutes and dilute to 25.0 ml with *water.*

Reference solution (a). A 0.025 per cent w/v solution of *bumetanide IPRS* in the solvent mixture. Dilute 10.0 ml of the solution to 25.0 ml with *water.*

Reference solution (b). A 0.0125 per cent w/v solution of *3-amino-4-phenoxy-5-sulphamoylbenzoic acid IPRS* in reference solution (a).

Chromatographic system

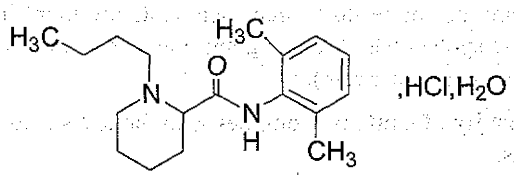
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *tetrahydrofuran*, 45 volumes of *water* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to bumetanide and 3-amino-4-phenoxy-5-sulphamoylbenzoic acid is not less than 15.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{20}N_2O_5S$ in the tablets.

Bupivacaine Hydrochloride



$C_{18}H_{28}N_2O,HCl,H_2O$ Mol. Wt. 342.9

Bupivacaine Hydrochloride is (*RS*)-1-butyl-*N*-(2,6-dimethylphenyl)-2-piperidinecarboxamide hydrochloride monohydrate.

Bupivacaine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{28}N_2O,HCl$, calculated on the dried basis.

Category. Local anaesthetic.

Description. A white, crystalline powder or colourless crystals; almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrphotometry (2.4.6). Compare the spectrum with that obtained with *bupivacaine hydrochloride IPRS* or with the reference spectrum of bupivacaine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in *0.01 M hydrochloric acid* shows two absorption maxima at about 263 nm and 271 nm; absorbance at about 263 nm, about 0.70 and at about 271 nm, about 0.57.

C. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of *2 M sodium hydroxide* and shake with two quantities, each of 15 ml, of *ether*. Dry the combined ether extracts over *anhydrous sodium sulphate*, filter, evaporate the ether, recrystallise the residue from *ethanol (90 per cent)* and dry the residue at a pressure of 1.5 to 2.5 kPa. The melting range (2.4.21) of the residue is between 105° and 108° (2.4.21).

D. A 10 per cent w/v solution gives reaction (A) of chlorides (2.3.1).

Tests

Acidity or alkalinity. To 10 ml of a 2.0 per cent w/v solution in *carbon dioxide-free water* add 0.2 ml of *0.01 M sodium hydroxide*; the pH is not less than 4.7. Add 0.4 ml of *0.01 M hydrochloric acid*; the pH is not more than 4.7 (2.4.24).

Appearance of solution. A 2.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

Related substances. Determine by gas chromatography (2.4.13).

Internal standard solution. Dissolve 25 mg of *methyl behenate* in *dichloromethane* and dilute to 500.0 ml with *dichloromethane*.

Test solution. Dissolve 50 mg of the substance under examination in 2.5 ml of *water*, add 2.5 ml of *dilute sodium hydroxide solution* and extract with 2 quantities, each of 5 ml, of the internal standard solution. Filter the lower layer.

Reference solution (a). Dissolve 10 mg each of the substance under examination, *bupivacaine impurity B IPRS* and

bupivacaine impurity E IPRS in 2.5 ml of *water*, add 2.5 ml of *dilute sodium hydroxide solution* and extract with 2 quantities, each of 5 ml, of the internal standard solution. Filter the lower layer and dilute to 20.0 ml with the internal standard solution.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the internal standard solution.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 10.0 ml with the internal standard solution.

Reference solution (d). Dilute 1.0 ml of reference solution (b) to 10.0 ml with the internal standard solution.

Chromatographic system

- a fused silica column 30 m x 0.32 mm, packed with poly(dimethyl)(diphenyl)siloxane (film thickness 0.25 µm),
- temperature:

column	time (min.)	temperature (°)
	0	180
	10	230
	15	230

- inlet port and detector at 250°,
- split ratio. 1:12,
- flame ionization detector,
- flow rate: 2.5 ml per minute, using nitrogen as the carrier gas.

Name	Relative retention time
Bupivacaine impurity C[1]	0.5
Bupivacaine impurity A[2]	0.6
Bupivacaine impurity B[3]	0.7
Bupivacaine impurity D[4]	0.8
Bupivacaine (Retention time: about 10 minutes)	1.0
Bupivacaine impurity E[5]	1.1
Methyl behenate (Internal Standard)	1.4

[1] 1-(2,6-dimethylphenyl)-1,5,6,7-tetrahydro-2*H*-azepin-2-one,

[2] *N*-(2,6-dimethylphenyl)pyridine-2-carboxamide,

[3] (2RS)-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide,

[4] (2RS)-2,6-dichloro-*N*-(2,6-dimethylphenyl)hexanamide,

[5] 6-(butylamino)-*N*-(2,6-dimethylphenyl)hexanamide.

Inject 1 µl of reference solution (a). The test is not valid unless the resolution between the peaks corresponding to bupivacaine and bupivacaine impurity E is not less than 3.0.

Inject 1 µl each of reference solution (b), (c), (d) and the test solution.

Bupivacaine impurity B. Calculate the ratio (*R*) of the area of the principal peak to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (c); from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to bupivacaine impurity B to the area of the peak due to the internal standard. This ratio is not more than *R* (0.5 per cent).

Any other secondary peak. Calculate the ratio (*R*) of the area of the principal peak to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (d); from the chromatogram obtained with the test solution, calculate the ratio of the area of any secondary peak other than the principal peak, bupivacaine impurity B peak and internal standard peak, to the area of the peak due to the internal standard. This ratio is not more than *R* (0.1 per cent).

The sum of all the secondary peaks. Calculate the ratio (*R*) of the area of the principal peak to the area of the peak due to the internal standard from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the sum of the areas of all the secondary peaks, to the area of the peak due to the internal standard. This ratio is not more than *R* (1.0 per cent). Ignore any peak with a ratio less than 0.01 times of *R* (0.01 per cent).

2,6-Dimethylaniline. To 2.0 ml of a 5.0 per cent w/v solution in *methanol* (solution A) add 1 ml of a freshly prepared 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand for 10 minutes. Any yellow colour produced is not more intense than that obtained with a solution prepared at the same time and in the same manner using 2 ml of a 0.0005 per cent w/v solution of *2,6-dimethylaniline* in *methanol* in place of solution A (100 ppm).

Heavy metals (2.3.13). A 10.0 per cent w/v solution in a mixture of 85 volumes of *methanol* and 15 volumes of *water* complies with the limit test for heavy metals Method D (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb)* obtained by diluting *lead standard solution (20 ppm Pb)* with a mixture of 85 volumes of *methanol* and 15 volumes of *water*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 4.5 to 6.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.25 g, dissolve in a mixture of 5.0 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (95 per cent)* and titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Note the volume added between the inflections.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.03249 g of $C_{18}H_{28}N_2O,HCl$.

Storage. Store protected from light.

Bupivacaine Injection

Bupivacaine Hydrochloride Injection

Bupivacaine Injection is a sterile solution of Bupivacaine Hydrochloride in Water for Injection.

Bupivacaine Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of anhydrous bupivacaine hydrochloride, $C_{18}H_{28}N_2O,HCl$.

Usual strengths. The equivalent of 25, 50 and 75 mg of anhydrous bupivacaine hydrochloride in 10 ml.

Identification

A. To a volume containing 25 mg of anhydrous bupivacaine hydrochloride add 2 ml of *strong ammonia solution*, shake and filter. Wash the precipitate with *water* and dry at 60° at a pressure of 2 kPa for 16 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bupivacaine hydrochloride IPRS* treated in the same manner or with the reference spectrum of bupivacaine.

B. To a volume containing 50 mg of anhydrous bupivacaine hydrochloride add 2 ml of a 10 per cent w/v solution of *disodium hydrogen phosphate* and sufficient *iodine solution* to produce a distinct brown colour. Remove the excess iodine by adding *0.1 M sodium thiosulphate*; no pink colour is produced.

Tests

pH (2.4.24). 4.0 to 6.5.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *methanol* and 0.1 volume of *strong ammonia solution*.

Test solution. Evaporate almost to dryness a volume containing 0.1 g of anhydrous bupivacaine hydrochloride using a rotary evaporator, add sufficient *methanol* to the residue to produce 2 ml, mix well, centrifuge and use the supernatant liquid.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

2,6-Dimethylaniline. To a volume containing 25 mg of anhydrous bupivacaine hydrochloride add *water*, if necessary, to produce 10 ml and sufficient *2 M sodium hydroxide* to make the solution just alkaline. Extract with three quantities, each of 5 ml, of *chloroform*. Dry the combined extracts over anhydrous *sodium sulphate*, filter, wash the filter with 5 ml of *chloroform* and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in 2 ml of *methanol*. Add 1 ml of a freshly prepared 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand for 10 minutes. Any yellow colour produced is not more intense than that obtained with a solution prepared at the same time and in the same manner using 2 ml of a 0.0005 per cent w/v solution of *2,6-dimethylaniline* in *methanol* in place of the injection under examination.

Bacterial endotoxins (2.2.3). Not more than 2.5 Endotoxin Units per mg of bupivacaine hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a quantity of the injection with sufficient mobile phase to produce a solution containing 0.0025 per cent w/v of anhydrous bupivacaine hydrochloride.

Reference solution (a). A 0.0025 per cent w/v solution of *bupivacaine hydrochloride IPRS* in the mobile phase.

Reference solution (b). A 0.1 per cent w/v solution of *2,6-dimethylaniline* in *acetonitrile*, dilute 10 volumes to 20 volumes with the mobile phase and then dilute 1 volume of the resulting solution to 100 volumes with reference solution (a).

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm), (Such as µBondapak C_{18})
- mobile phase: a mixture of 40 volumes of *0.02M phosphate buffer pH 8.0* and 60 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to bupivacaine hydrochloride and 2,6-dimethylaniline is not less than 8.0.

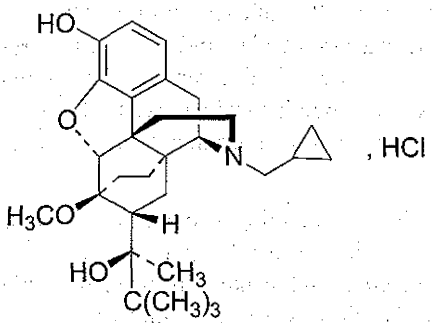
Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{28}N_2O,HCl$.

Storage. Store in single dose or multiple dose containers, preferably of Type 1 glass.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous bupivacaine hydrochloride in a suitable dose-volume.

Buprenorphine Hydrochloride



$C_{29}H_{41}NO_4,HCl$ Mol. Wt. 504.1

Buprenorphine Hydrochloride is (6*R*,7*R*,14*S*)-17-cyclopropylmethyl-7,8-dihydro-7-[(1*S*)-1-hydroxy-1,2,2-trimethylpropyl]-6-*O*-methyl-6,14-ethano-17-normorphine hydrochloride.

Buprenorphine Hydrochloride contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{29}H_{41}NO_4,HCl$, calculated on the dried basis.

Category. Narcotic analgesic.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *buprenorphine hydrochloride IPRS* or with the reference spectrum of buprenorphine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum at about 286 nm, about 0.33.

C. Dissolve about 5 mg in 5 ml of hot *water*, add 2 ml of *dilute hydrochloric acid* and 2 ml of a 2 per cent w/v solution of *sodium nitrite* and allow to stand for 10 minutes; a yellow colour is produced.

D. Dissolve 10 mg in 10 ml of hot *water*, add 2 ml of *dilute nitric acid*, shake and add 1 ml of *silver nitrate solution*; a white precipitate is produced.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 1.0 ml of the solution to 10.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile* and 90 volumes of a 0.544 per cent w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 4.5 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	89	11
2	89	11
12	64	36
15	41	59
20	39	61
21	89	11
30	89	11

Name	Relative retention time	Correction factor
Buprenorphine impurity B[1]	0.4	—
Buprenorphine Hydrochloride (Retention time: about 8.5 minutes)	1.0	---
Buprenorphine impurity J[2]	1.1	—
Buprenorphine impurity F[3]	1.27	---
Buprenorphine impurity H[4]	1.33	---
Buprenorphine impurity A[5]	1.4	—
Buprenorphine impurity G[6]	1.8	0.3

[1]norbuprenorphine,

[2](2*S*)-2-[17-(cyclopropylmethyl)-4,5α -epoxy-3-hydroxy-6-methoxy-6α,14- etheno-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol,

[3]17-(cyclopropylmethyl)-4,5α-epoxy-6-methoxy-7α-[1-(1,1-dimethylethyl) ethenyl]-6α,14-ethano-14α-morphinan-3-ol,

[4](2*S*)-2-[17-butyl-4,5 α-epoxy-3-hydroxy-6-methoxy-6α, 14-ethano-14α-morphinan-7α-yl]3,3-dimethylbutan-2-ol,

[5](2*S*)-2-[17-(but-3-enyl)-4,5α-epoxy-3-hydroxy-6- methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol,

[6]2,2'-bibuprenorphine.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the

principal peak in the chromatogram obtained with the reference solution (0.25 per cent). The sum of the areas of all the secondary peaks is not more than 7 times the area of the principal peak in the chromatogram obtained with the reference solution (0.7 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 4 hours.

Assay. Weigh 0.5 g, dissolve in 60 ml of *anhydrous glacial acetic acid*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using 0.1 ml of *crystal violet solution* as indicator to a green end-point. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05041 g of $C_{29}H_{41}NO_4,HCl$.

Buprenorphine Injection

Buprenorphine Hydrochloride Injection

Buprenorphine Injection is a sterile solution of Buprenorphine Hydrochloride in Water for Injection.

Buprenorphine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of buprenorphine, $C_{29}H_{41}NO_4$.

Usual strength. The equivalent of 300 µg of buprenorphine per ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 85 volumes of *toluene*, 15 volumes of *methanol* and 0.5 volume of *strong ammonia solution*.

Test solution. Transfer a volume of the injection containing 1.5 mg of Buprenorphine Hydrochloride to a 125-ml separator, add 0.5 ml of *dilute ammonia solution*, shake and extract with three quantities, each of 10 ml, of *chloroform*, washing each chloroform extract with the same 10 ml of *water* and discard the *water*. Evaporate the combined chloroform extracts to dryness on a water-bath and dissolve the residue in 1.5 ml of *chloroform*.

Reference solution. Dissolve 1.5 mg of *buprenorphine hydrochloride IPRS* in 5 ml of *0.01 M hydrochloric acid*, transfer the solution to a 125-ml separator and repeat the above procedure beginning at the words "add 0.5 ml of *dilute ammonia solution*.....".

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm or expose to iodine vapours. The principal spot in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. To a volume containing about 5 mg of Buprenorphine Hydrochloride in a 125-ml separator, add 1 ml of *dilute ammonia solution* and shake with three quantities, each of 10 ml, of *chloroform*. Wash each chloroform extract with the same 10 ml of *water* and discard the washings. Evaporate the combined chloroform extracts to dryness on a water-bath and dissolve the residue in 50 ml of *0.1 M hydrochloric acid*. When examined in the range 230 to 360 nm (2.4.7) the resulting solution shows an absorption maximum only at about 286 nm.

Tests

pH (2.4.24). 3.5 to 6.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a quantity of the injection, if necessary, with *methanol* to obtain a solution containing 0.03 per cent w/v of buprenorphine.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Further dilute 1.0 ml of the solution to 2.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile* and 90 volumes of a 0.544 per cent w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 4.5 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	89	11
2	89	11
12	64	36
15	41	59
20	39	61
21	89	11
30	89	11

Name	Relative retention time	Correction factor
Buprenorphine impurity B[1]	0.4	—
Buprenorphine Hydrochloride (Retention time: about 8.5 minutes)	1.0	—
Buprenorphine impurity J[2]	1.1	—
Buprenorphine impurity F[3]	1.27	—
Buprenorphine impurity H[4]	1.33	—
Buprenorphine impurity A[5]	1.4	—
Buprenorphine impurity G[6]	1.8	0.3

[1]norbuprenorphine,

[2](2*S*)-2-[17-(cyclopropylmethyl)-4,5α -epoxy-3-hydroxy-6-methoxy-6α,14- etheno-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol,

[3]17-(cyclopropylmethyl)-4,5α-epoxy-6-methoxy-7α-[1-(1,1-dimethylethyl) ethenyl]-6α,14-ethano-14α-morphinan-3-ol,

[4](2*S*)-2-[17-butyl-4,5α-epoxy-3-hydroxy-6-methoxy-6α, 14-ethano-14α-morphinan-7α-yl]3,3-dimethylbutan-2-ol,

[5](2*S*)-2-[17-(but-3-enyl)-4,5α-epoxy-3-hydroxy-6- methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol,

[6]2,2'-bibuprenorphine.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Measure a volume containing 1.5 mg of buprenorphine and transfer to a 25-ml volumetric flask. Add 1 ml of *1 M hydrochloric acid*, 2 ml of a 2 per cent w/v solution of *sodium nitrite* and shake well. Stopper the flask and allow to stand for 15 minutes. Dilute the solution to volume with *dilute ammonia solution* and measure the absorbance of the resulting solution at the maximum at about 460 nm (2.4.7), using as the blank a solution prepared in the same manner by treating 5 ml of *water* instead of the preparation under examination.

Calculate the content of $C_{29}H_{41}NO_4$ from the absorbance obtained by repeating the procedure with 5 ml of a solution containing *buprenorphine hydrochloride IPRS* equivalent to 0.03 per cent w/v of buprenorphine.

Labelling. The label states the strength in terms of the equivalent amount of buprenorphine in a suitable dose-volume.

Buprenorphine Sublingual Tablets

Buprenorphine Hydrochloride Tablets

Buprenorphine Sublingual Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of buprenorphine, $C_{29}H_{41}NO_4$.

Usual strengths. The equivalent of 200 µg and 400 µg of buprenorphine .

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Evaporate 10 ml of the test solution obtained in the Assay to dryness. Dissolve the residue in 5 ml of *water*, add sufficient amount of *dilute hydrochloric acid* to turn litmus paper red and add 1 ml of *potassium iodobismuthate solution*. An orange red precipitate is formed.

Tests

Disintegration (2.5.1). Not more than 2 minutes.

Related substances. Determine by liquid chromatography (2.4.14).

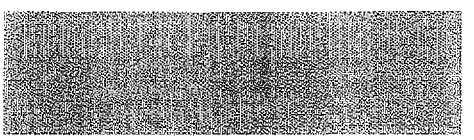
Test solution. Disperse a quantity of the powdered tablets containing 4 mg of buprenorphine with 4 ml of *methanol* with the aid of ultrasound and filter.

Reference solution. Dilute 1.0 ml of the test solution to 50.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as Sunfire C18),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile* and 90 volumes of a 0.544 per cent w/v solution of *potassium dihydrogen orthophosphate* previously adjusted to pH 4.5 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	89	11
2	89	11
12	64	36
15	41	59
20	39	61
21	89	11
30	89	11



Name	Relative retention time	Correction factor
Buprenorphine impurity B[1]	0.4	—
Buprenorphine Hydrochloride (Retention time: about 8.5 minutes)	1.0	---
Buprenorphine impurity J[2]	1.1	---
Buprenorphine impurity F[3]	1.27	---
Buprenorphine impurity H[4]	1.33	---
Buprenorphine impurity A[5]	1.4	---
Buprenorphine impurity G[6]	1.8	0.3

[1]norbuprenorphine,

[2](2*S*)-2-[17-(cyclopropylmethyl)-4,5α -epoxy-3-hydroxy-6-methoxy-6α,14- etheno-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol,

[3]17-(cyclopropylmethyl)-4,5α-epoxy-6-methoxy-7α-[1-(1,1-dimethylethyl) ethenyl]-6α,14-ethano-14α-morphinan-3-ol,

[4](2*S*)-2-[17-butyl-4,5 α-epoxy-3-hydroxy-6-methoxy-6α, 14-ethano-14α-morphinan-7α-yl]3,3-dimethylbutan-2-ol,

[5](2*S*)-2-[17-(but-3-enyl)-4,5α-epoxy-3-hydroxy-6- methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol,

[6]2,2'-bibuprenorphine.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). The area of not more than one secondary peak is more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). The sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (6.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Related substances using 25 µl injection volume and with the following modifications.

Test solution. Disperse 1 tablet in 1 ml of *methanol*, with the aid of ultrasound and dilute with the mobile phase to obtain a solution containing 0.004 per cent w/v of buprenorphine.

Reference solution. A 0.004 per cent w/v solution of *buprenorphine hydrochloride IPRS* in *methanol*.

Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{41}NO_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 2 mg of buprenorphine in *methanol*, with the aid of ultrasound and dilute to 20.0 ml with *methanol*.

Reference solution. A 0.01 per cent w/v solution of *buprenorphine hydrochloride IPRS* in *methanol*.

Chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{41}NO_4$ in the tablets.

1 mg of $C_{29}H_{41}NO_4$,HCl is equivalent to 0.9276 mg of $C_{29}H_{41}NO_4$.

Labelling. The label states the strength in terms of the equivalent amount of buprenorphine.

Buprenorphine and Naloxone Sublingual Tablets

Buprenorphine Hydrochloride and Naloxone Hydrochloride Sublingual Tablets

Buprenorphine and Naloxone Sublingual Tablets contain Buprenorphine Hydrochloride and Naloxone Hydrochloride equivalent to not less than 90.0 per cent and not more than 110.0 per cent of stated amount of buprenorphine, $C_{29}H_{41}NO_4$ and naloxone, $C_{19}H_{21}NO_4$.

Usual strengths. Buprenorphine, 0.4 mg and Naloxone, 0.1 mg; Buprenorphine, 2 mg and Naloxone, 0.5 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 500 ml of *water*,
Speed and Time: 100 rpm and 10 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 2.4 g of *monobasic potassium phosphate* and 0.5 g of *sodium hydroxide* in 1000 ml of *water*, adjusted to pH 6.8 with *orthophosporic acid.*

Solvent mixture. Equal volumes of *water* and *methanol.*

Test solution. Use the filtrate, dilute, if necessary, with the dissolution medium.

Reference solution. Dissolve a suitable quantity of *buprenorphine hydrochloride IPRS* and *naloxone hydrochloride IPRS* in the solvent mixture and dilute with the dissolution medium to obtain a solution of the similar concentration as that of the test solution.

Chromatographic system

- a stainless steel column 50 mm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 40 volumes of *acetonitrile,* 20 volumes of *methanol* and 40 volumes of the buffer solution,
  B. a mixture of 78 volumes of *acetonitrile,* and 22 volumes of the buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 40 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
2	100	0
3	0	100
6	0	100
6.1	100	0
8	100	0

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for buprenorphine and naloxone peaks.

Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{41}NO_4$ and $C_{19}H_{21}NO_4$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{29}H_{41}NO_4$ and $C_{19}H_{21}NO_4$.

Related substances. Determine by liquid chromatography (2.4.14), using chromatographic system, buffer solution, solvent mixture and test solution as described under Assay.

Reference solution. A solution containing 0.00016 per cent w/v of *buprenorphine hydrochloride IPRS* and 0.00005 per cent w/v of *naloxone hydrochloride IPRS* in the solvent mixture.

Name	Relative retention time
Naloxone degradation product 1[1]	0.30
Naloxone degradation product 2[1]	0.54
Dealkyl buprenorphine[2,3]	0.55
Naloxone	0.61
Naloxone degradation product 3[1]	0.67
Buprenorphine nitrile[3,4]	0.90
6-O-desmethylbuprenorphine[3,5]	0.91
Buprenorphine degradation product 1[6]	0.95
Buprenorphine 7-(S)-epimer[3,7]	0.99
Buprenorphine	1.00
Buprenorphine butenyl analog[3,8]	1.03
3-O-Methylbuprenorphine[3,9]	1.16

[1]Quantified relative to naloxone,

[2](S)-2-(4,5α-Epoxy-3-hydroxy-6-methoxy-6α,14-ethanomorphinan-7α-yl)-3,3-dimethylbutan-2-ol,

[3]These are process impurities and are excluded from the total degradation products,

[4]4,5α-Epoxy-7α-[(S)-2-hydroxy-3,3-dimethylbutan-2-yl]-3,6-dimethoxy-6α,14-ethanomorphinan-17-carbonitrile,

[5](S)-2-[17-(Cyclopropylmethyl)-4,5α-epoxy-3,6-dihydroxy-6α,14-ethanomorphinan-7α-yl]-3,3-dimethylbutan-2-ol,

[6]Quantified relative to buprenorphine,

[7](S)-2-[17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethanomorphinan-7α-yl]-3,3-dimethylbutan-2-ol,

[8](S)-2-[17-(But-3-en-1-yl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethanomorphinan-7α-yl]-3,3-dimethylbutan-2-ol,

[9](S)-2-[17-(Cyclopropylmethyl)-4,5α-epoxy-3,6-dimethoxy-6α,14-ethanomorphinan-7α-yl]-3,3-dimethylbutan-2-ol.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent for both the peaks.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to naloxone degradation product 1, 2 and 3, each of, is not more than 5 times the area of the naloxone peak in the chromatogram obtained with the reference solution (0.5 per cent), the area of any peak corresponding to buprenorphine degradation product 1 is not more than the area of the buprenorphine peak in the chromatogram obtained with the reference solution (0.3 per cent). The area of any other secondary peak is not more than 3 times the area of the naloxone peak in the chromatogram obtained with the reference solution (0.3 per cent) and the sum of areas of all the secondary peaks excluding process impurities is not more than 30 times the area of the naloxone peak in the chromatogram obtained with the reference solution (3.0 per cent). Ignore any peak with an area less than 0.5 times the area of the naloxone peak

in the chromatogram obtained with the reference solution (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse one tablet in the solvent mixture and dilute with the solvent mixture to obtain a solution of the similar concentration as that of the reference solution.

Reference solution. A solution containing 0.0023 per cent w/v of *buprenorphine hydrochloride IPRS* and 0.00056 per cent w/v of *naloxone hydrochloride IPRS* in the solvent mixture.

Calculate the content of the $C_{29}H_{41}NO_4$ and $C_{19}H_{21}NO_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 7 volumes of *acetonitrile,*3 volumes of *methanol,* and 90 volumes of 0.1 per cent v/v solution of *orthophosporic acid.*

Buffer solution. A *0.009 M dibasic ammonium phosphate buffer,* adjusted to pH 6.2 with 50 per cent v/v solution of *orthophosporic acid.*

NOTE — Protect all solution from light.

Test solution. Disperse a suitable numbers of intact tablets (not less than 13 tablets) in 35 ml of the solvent mixture with the aid of ultrasound for 15 minutes with occasional swirling and shake for 15 minutes, dilute with the solvent mixture to obtain a solution containing 0.052 per cent w/v of Buprenorphine. Centrifuge at 3000 rpm for 10 minutes and use supernatant.

Reference solution. A solution containing 0.057 per cent w/v of *buprenorphine hydrochloride IPRS* and 0.016 per cent w/v of *naloxone hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 mm × 4.6 mm, packed with phenyl group bonded to porous silica (5 µm),
- column temperature: 60°,
- mobile phase: A. a mixture of 7 volumes of *acetonitrile,* 3 volumes of *methanol* and 90 volumes of the buffer solution,

  B. a mixture of 56 volumes of *acetonitrile,* 24 volumes of *methanol* and 20 volumes of the buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	99	1
30	1	99
45	1	99
45.1	99	1
55	99	1

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviationn for replicate injections is not more than 2.0 per cent for both the peaks.

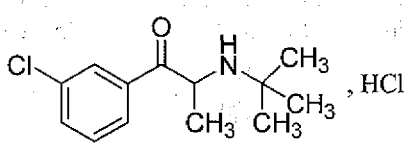
Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{41}NO_4$ and $C_{19}H_{21}NO_4$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The quantity of active ingredients is stated in terms of the equivalent amount of Buprenorphine and Naloxone.

Bupropion Hydrochloride



$C_{13}H_{18}ClNO,HCl$ Mol. Wt. 276.2

Bupropion Hydrochloride is 1-propanone,1-(3-chlorophenyl)-2-[(1,1-dimethylethyl) amino]-, hydrochloride, (±) -;(±) -2- (tert-butyl amino)-3′-chloropropiophenone hydrochloride.

Bupropion Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{13}H_{18}ClNO,HCl$, calculated on the anhydrous basis.

Category. Anti depressant.

Description. A white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained from *bupropion hydrochloride IPRS* or with the reference spectrum of bupropion hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Gives the reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Use the test solution, reference solution and chromatographic system as described under Assay.

Name	Relative retention time	Correction factor
Deschlorobupropion[1]	0.38	0.66
Bupropion dione derivative[2]	0.58	1.0
O-Bupropion[3]	0.71	2.22
Chloropropiophenone[4]	0.78	0.83
Bupropion hydrochloride related compound A	0.92	0.71
Bupropion	1.0	—
Bupropion hydrochloride related compound B	1.14	1.23
Bromochloropropionphenone[5]	1.63	1.13
4-Chlorobupropion[6]	2.30	0.90
5-Chlorobupropion[7]	2.74	1.44
Unknown impurity	—	1.0

[1]2-(tert-butylamino)-1-phenylpropan-1-one; 2-(tert-butylamino) propiophenone,

[2]1-(3-chlorophenyl) propane-1,2-dione; 1-(3-chlorophenyl) -1,2-propanedione,

[3]2-(tert-butylamino)-1-(2-chlorophenyl) propan-1-one; 2-(tert-butylamino)-2'-chloropropiophenone,

[4]1-(3- chlorophenyl) propan-1-one; 3'-chloropropiophenone,

[5]2-Bromo-1-(3-chlorophenyl) propan-1-one; 2-bromo-3'-chloropropiophenone,

[6]2-(tert-butylamino)1-(3,4-dichlorophenyl) propan-1-one ; 2-(tert-butylamino-3',4'-dichloropropiophenone,

[7]2-(tert-butylamino)-1-(3,5-dichlorophenyl) propan-1-one; 2-(tert-butylamino)-3',5'-dichloropropiophenone.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to bupropion hydrochloride related compound A and bupropion is not less than 1.3 and resolution between the peaks due to bupropion and bupropion hydrochloride related compound B is not less than 1.3. The relative standard deviation for bupropion is not more than 2.0 per cent and bupropion hydrochloride related compound B is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of deschloro bupropion peak is not more than 0.005 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The area of bupropion dione derivative, bupropion hydrochloride related compound A, bupropion hydrochloride related compound B, 4-chlorobupropion and 5- chlorobupropion peak is not more than 0.002 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). The area of o-bupropion, chloropropiophenone, bromochloropropionphenone and any other single impurity peak is not more than 0.001 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent). The sum of the areas of all the secondary peaks is not more than 0.01 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Limit of 3-Chlorobenzoic Acid. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *methanol* and 80 volumes of *0.001M hydrochloric acid*.

NOTE — Prepare the solutions immediately before use and protect from light.

Test solution. Dissolve 60 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution(a). A solution containing 0.002 per cent w/v of *bupropion hydrochloride related compound C IPRS (1-(3-chlorophenyl)-2-hydroxypropan-1-one), bupropion hydrochloride related compound F IPRS((1-(3-chlorophenyl)-1-hydroxypropan-2-one)* and 0.0012 per cent w/v of *3-chlorobenzoic acid IPRS* in *methanol*.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A 0.012 per cent w/v solution of *3-chlorobenzoic acid IPRS* in *methanol*. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 100 volumes of *acetonitrile* and 900 volumes of *water*, add 0.4 ml of *trifluoro acetic acid*,

  B. a mixture of 950 volumes of *acetonitrile* and 50 volumes of *water*, add 0.3 ml of *trifluoro acetic acid*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
3.4	87	13
10.0	15	85
10.1	0	100
13.0	0	100
13.2	90	10
19.0	90	10

Name	Relative retention time
Bupropion	1.0
Bupropion hydrochloride related compound F	1.71
Bupropion hydrochloride related compound C	1.75
3- Chlorobenzoic acid	1.80

Inject reference solution (b). The test is not valid unless the resolution between bupropion hydrochloride related compound F and bupropion hydrochloride related compound C is not less than 1.5 and the resolution between bupropion hydrochloride related compound C and 3-chlorobenzoic acid is not less than 1.3. The relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of 3-chlorobenzoic acid peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent).

Water (2.3.43). Not more than 0.5 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *methanol* and 50 volumes of *water*.

Test solution. Dissolve 100 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution. A solution containing 0.1 per cent w/v of *bupropion hydrochloride IPRS* and 0.0002 per cent w/v each of *bupropion hydrochloride related compound A IPRS (2-tert-butylamino)-4'-chloropropiophenone hydrochloride)* and *bupropion hydrochloride related compound B IPRS (2-tert-butylamino)3'bromopropiophenone hydrochloride)* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 3.9 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 3.4 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 7.0 with *1 M sodium hydroxide solution*, and diluting to 1000 ml with *water*, 11 volumes of *tetrahydrofuran* and 39 volumes of *methanol*,
- flow rate: 1.1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to bupropion hydrochloride related compound A and bupropion is not less than 1.3, the resolution between the peaks due to bupropion hydrochloride related compound B and bupropion is not less than 1.3 . The relative standard deviation for replicate injections for the peak due to bupropion is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{18}ClNO$, HCl.

Storage. Store protected from light and moisture, at temperature not exceeding 30°.

Bupropion Hydrochloride Prolonged-release Tablets

Bupropion Hydrochloride Sustained-release Tablets; Bupropion Hydrochloride Extended-release Tablets

Bupropion Hydrochloride Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Bupropion Hydrochloride Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of bupropion hydrochloride, $C_{13}H_{18}ClNO$, HCl.

Usual strengths. 100 mg; 150 mg; 300 mg.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *bupropion hydrochloride IPRS* or with the reference spectrum of bupropion hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (a) or (b) corresponds to the peak in the chromatogram obtained with reference solution (g).

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture A. 20 volumes of *methanol* and 80 volumes of *0.001 M hydrochloric acid*.

Solvent mixture B. a mixture of 80 volumes of buffer solution prepared by dissolving 100 g of *anhydrous disodium hydrogen phosphate* in 1000 ml of *water*. Add 50 ml of *orthophosphoric acid* with the aid of ultrasound and mix. Adjusted to pH 3.0 with *orthophosphoric acid* and 20 volumes of *methanol*.

Test solution (a). Disperse a quantity of the powdered tablets, containing 300 mg of Bupropion Hydrochloride in 100.0 ml of *methanol* with the aid of ultrasound and centrifuge at

20000 rpm for about 3 minutes. Filter, discarding the first few ml of filtrate. Dilute 10.0 ml of the solution to 50.0 ml with *0.001 M hydrochloric acid*.

NOTE—Alternatively, the sample can be prepared as follows.

Test solution (b). Weigh and powder 20 tablets. Disperse a quantity of powder containing 300 mg of Bupropion Hydrochloride in 75 ml of solvent mixture (b). Stir for 30 minutes and ultrasound for 15 minutes and dilute to 100.0 ml with the same solvent and centrifuge, use the supernatant. Dilute 10.0 ml of the solution to 50.0 ml with solvent mixture B.

Reference solution (a). A solution containing 0.002 per cent w/v of *bupropion hydrochloride C IPRS* [1-(3-chlorophenyl)-2-hydroxypropan-1-one], *bupropion hydrochloride F IPRS* [1-(3-chlorophenyl)-1-hydroxypropan-2-one] and 0.0012 per cent w/v of *3-chlorobenzoic acid IPRS* in *methanol*.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 100.0 ml with solvent mixture A.

Reference solution (c). A 0.0012 per cent w/v solution of *3-chlorobenzoic acid IPRS* in *methanol*.

Reference solution (d). Dilute 10.0 ml of reference solution (c) to 100.0 ml with solvent mixture A.

Reference solution (e). A 0.00012 per cent w/v solution of *bupropion hydrochloride IPRS* in the solvent mixture A.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 10 volumes of *acetonitrile*, 0.04 volume of *trifluoroacetic acid* and 90 volumes of *water*,
  B. a mixture of 95 volumes of *acetonitrile*, 0.03 volume of *trifluoroacetic acid* and 5 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 226 ± 2 nm so that the correction factor requirement is met,
  (*NOTE — The peak responses of the compounds of interest are very sensitive to change in the detection wave length*),
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
3.4	87	13
10.0	15	85
10.1	0	100
13.0	0	100
13.2	90	10
19.0	90	10

Name	Relative retention time	Correction factor
Bupropion amine[1]	0.38	0.83
S,S,S-Thiomorpholine derivative[2]	0.56	0.90
S,R,R-Thiomorpholine derivative[3]	0.78	0.90
Bupropion	1.0	—
Bupropion related compound F	1.71	0.55
Bupropion related compound C	1.75	0.58
3-Chlorobenzoic acid	1.80	—
Bupropion dione derivative[4]	2.25	1.0
Unknown impurity	---	1.0

[1]2-Amino-1-(3-chlorophenyl)-1-propanone,

[2](3*S*,5*S*,6*S*)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid,

[3](3*S*,5*R*,6*R*)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid,

[4]1-(3-chlorophenyl) propane-1,2-dione.

Inject reference solution (b), (d) and (e). The test is not valid unless the resolution between the peaks due to bupropion hydrochloride related compound F and bupropion hydrochloride related compound C is not less than 1.3 and peak due to bupropion hydrochloride related compound C and 3-chlorobenzoic acid is not less than 1.3 in the chromatogram obtained with reference solution (b) and relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (e).

For 100 mg or less —

Inject reference solution (c), (e) and test solution (a) or (b). In the chromatogram obtained with test solution (a) or (b), the area of any peak due to bupropion amine is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.3 per cent), the area of the any peak due to S,S,S-thiomorpholine derivative is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (1.0 per cent), the area of any peak due to S,R,R-thiomorpholine derivative is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.5 per cent), the area of any peak due to bupropion related compound F is not more than 6.0 times the area of the principal peak in the chromatogram obtained with reference solution (e) (1.2 per cent), the area of any peak due to bupropion related compound C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.3 per cent), the area of any peak due to 3- chlorobenzoic acid is not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent), the area of any peak due to bupropion dione

derivative is not more than 2.0 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.4 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent). The sum of areas of all the secondary peaks is not more than 16 times the area of the principal peak in the chromatogram obtained with reference solution (e) (3.2 per cent).

For 150 mg or more —

Inject reference solution (c), (e) and test solution (a) or (b). In the chromatogram obtained with test solution (a) or (b), the area of any peak due to bupropion amine is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.3 per cent), the area of any peak due to S,S,S-thiomorpholine derivative is not more than 7.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (1.5 per cent), the area of any peak due to S,R,R-thiomorpholine derivative is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (e) (0.4 per cent),the area of any peak due to bupropion related compound F is not more than 11.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (2.3 per cent),the area of any peak due to bupropion related compound C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.3 per cent), the area of any peak due to 3- chlorobenzoic acid is not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent),the area of any peak due to bupropion dione derivative is not more than 2.0 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.4 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent). The sum of areas of all the secondary peaks is not more than 16.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (3.3 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14). Use Solvent mixture (a), mobile phase (a) and (b), test solution (a) or test solution (b) as described under Related substances.

Reference solution (f). A solution containing 0.002 per cent w/v of *bupropion hydrochloride C IPRS* [1-(3-chlorophenyl)-2-hydroxypropan-1-one] and 0.02 per cent w/v of *bupropion hydrochloride F IPRS* [1-(3-chlorophenyl)-1-hydroxypropan-2-one] in *methanol*. Dilute 10.0 ml of the solution to 100.0 ml with solvent mixture (a).

Reference solution (g). A 0.06 per cent w/v solution of *bupropion hydrochloride IPRS* in solvent mixture (a).

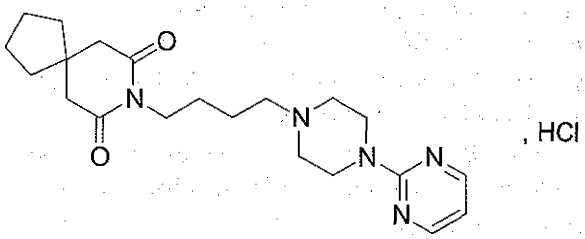
Inject reference solution (f) and (g). The test is not valid unless the resolution between the peaks due to bupropion hydrochloride related compound F and bupropion hydrochloride related compound C is not less than 1.3 in the chromatogram obtained with reference solution (f). The tailing factor is not more than 1.9 and the relative standard deviation for replicate injections is not more than 1.5 per cent in the chromatogram obtained with reference solution (g).

Inject reference solution (g) and test solution (a) or (b).

Calculate the content of $C_{13}H_{18}ClNO$, HCl in the tablets.

Storage. Store protected from moisture and at a temperature not exceeding 30°.

Buspirone Hydrochloride



$C_{21}H_{31}N_5O_2$,HCl Mol. Wt. 422.0

Buspirone Hydrochloride is 8-[4-[4-(pyrimidin-2-yl) piperazin-1-yl]butyl]-8-azaspiro[4.5]decane-7,9-dione hydrochloride.

Buspirone Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{31}N_5O_2$,HCl, calculated on the dried basis.

Category. Anxiolytic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *buspirone hydrochloride IPRS* or with the reference spectrum of buspirone hydrochloride. If the spectra obtained show differences, dissolve the substance under examination and the reference substance separately in *methanol,* evaporate to dryness on a water-bath and record new spectra using the residues.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution (b). A 0.05 per cent w/v solution of *buspirone impurity A IPRS (2,2' (piperazine-1, 4-diyl) dipyrimidine IPRS)* in the test solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. 95 volumes of a solution containing 0.68 per cent w/v of *potassium dihydrogen phosphate* and 0.093 per cent w/v of *sodium hexanesulphonate monohydrate*, previously adjusted to pH 3.4 with *orthophosphoric acid* and 5 volumes of *acetonitrile*,
  B. 25 volumes of a solution containing 0.34 per cent w/v of *potassium dihydrogen phosphate* and 0.352 per cent w/v of *sodium hexanesulphonate monohydrate*, previously adjusted to pH 2.2 with *orthophosphoric acid* and 75 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm and at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
6	90	10
34	42	58
45	42	58
55	0	100
56	100	0
60	100	0
61	90	10

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio at 240 nm is not less than 5.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to buspirone.

Inject reference solution (a) and the test solution and set the spectrophotometer at 240 nm. In the chromatogram obtained with the test solution, the area of any secondary peak obtained is not more than 3 times the area obtained with reference solution (a) (0.3 per cent). The sum of the areas of all the secondary peaks is not more than 5 times the area obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Inject reference solution (a) and the test solution and set the spectrophotometer at 210 nm. In the chromatogram obtained with the test solution, the area of any secondary peak obtained is not more than 3 times the area obtained with reference solution (a) (0.3 per cent). The sum of the areas of all the secondary peaks is not more than 5 times the area obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.15 g in 10 ml of *glacial acetic acid* and add 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0211 g of $C_{21}H_{32}ClN_5O_2$.

Storage. Store protected from light.

Buspirone Tablets

Buspirone Hydrochloride Tablets

Buspirone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of buspirone, $C_{21}H_{31}N_5O_2$.

Usual strengths. 5 mg; 10 mg.

Identification

A. Extract a quantity of the powdered tablets containing 50 mg of buspirone with 50 ml of *chloroform*, filter and evaporate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *buspirone hydrochloride IPRS* or with the reference spectrum of buspirone hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.01 M hydrochloric acid*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 235 nm (2.4.7). Calculate the content of $C_{21}H_{31}N_5O_2$ in the medium from the absorbance obtained from a solution of known concentration of *buspirone hydrochloride IPRS* in the same medium.

Q: Not less than 80 per cent of the stated amount of $C_{21}H_{31}N_5O_2$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions and the reference solution as described under Assay.

Test solution. Disperse one tablet in the minimum amount of *1 M hydrochloric acid* and dilute with water to produce a solution containing 0.005 per cent w/v of buspirone, shake and filter.

Calculate the content of $C_{21}H_{31}N_5O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 25 mg of buspirone, disperse in 15 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with *water*, filter. Dilute 10.0 ml of filtrate to 100.0 with *water*.

Reference solution. Dissolve 30 mg of *buspirone hydrochloride IPRS* in 15 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with *water*. Dilute 10.0 ml of the solution to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *methanol* and 35 volumes of *0.067 M monobasic potassium phosphate*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

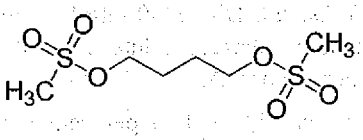
Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{31}N_5O_2$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of buspirone.

Busulphan



$C_6H_{14}O_6S_2$ Mol. Wt. 246.3

Busulphan is 1,4-butanediol dimethanesulphonate.

Busulphan contains not less than 99.0 per cent and not more than 100.5 per cent of $C_6H_{14}O_6S_2$, calculated on the dried basis.

Category. Anticancer.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *busulphan IPRS* or with the reference spectrum of busulphan.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of equal volumes of *acetone* and *toluene*.

Test solution. Dissolve 1 g of the substance under examination in 100 ml of *acetone*.

Reference solution. A 1 per cent w/v solution of *busulphan IPRS* in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of hot air, spray with *anisaldehyde solution* and heat at 120°. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Heat 0.1 g with 5 ml of *1 M sodium hydroxide* until a clear solution is obtained and allow to cool. To 2 ml of the solution add 0.1 ml of a 3 per cent w/v solution of *potassium permanganate*; the purple colour changes to violet, then to blue and finally to green. Filter and add 1 ml of *ammoniacal silver nitrate solution*; a precipitate is produced.

D. Fuse 0.1 g with 0.1 g of *potassium nitrate* and 0.25 g of *potassium hydroxide*, cool and dissolve the residue in 5 ml of *water*. Acidify with *dilute hydrochloric acid* and add a few drops of *barium chloride solution*; a white precipitate is produced.

Tests

Appearance of solution. Dissolve 0.25 g in 20.0 ml of *acetonitrile*, dilute to 25 ml with *water* and examine

immediately. The solution is clear (2.4.1), and not more intensely coloured than reference solution BS6 (2.4.1).

Acidity. Dissolve 0.2 g in 50 ml of warm *ethanol* previously neutralised to *methyl red solution* and titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator; not more than 0.05 ml of *0.1 M sodium hydroxide* is required.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure of 1.5 to 2.5 kPa.

Assay. Weigh 0.25 g and shake with 50 ml of *water*. Boil under a reflux condenser for 30 minutes and, if necessary, restore the initial volume with *water*. Allow to cool and titrate with *0.1 M sodium hydroxide*, using 0.3 ml of *dilute phenolphthalein solution* as indicator, until a pink colour is produced.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01232 g of $C_6H_{14}O_6S_2$.

Storage. Store protected from light.

Busulphan Tablets

Busulphan Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of busulphan, $C_6H_{14}O_6S_2$. The tablets are coated.

Usual strength. 2 mg.

Identification

A. Warm a quantity of the powdered tablets containing 10 mg of Busulphan with 10 ml of *acetone*, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *busulphan IPRS* or with the reference spectrum of busulphan.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution(b).

Tests

Disintegration (2.5.1). Maximum time, 15 minutes.

Uniformity of content. Complies with the test stated under Tablets.

Determine by gas chromatography (2.4.13).

Test solution. Add 1 ml of *water* to one tablet in a 50-ml volumetric flask and place in an ultrasonic bath until completely dispersed. Add 30 ml of *acetone*, shake for 15 minutes and dilute to 50.0 ml with *acetone*. Centrifuge and dilute a quantity of the supernatant liquid with *acetone* to produce a solution containing 0.0001 per cent w/v of Busulphan. To 5.0 ml of the resulting solution add 5 ml of a 30 per cent w/v solution of *sodium iodide* in *acetone*, stopper the flask lightly and heat in a water-bath at 50° for 90 minutes. Cool, add 10 ml of a 0.0001 per cent w/v solution of *1,5-di-iodopentane* (internal standard) in *acetone*, mix, add 10 ml of *water* and 20.0 ml of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

Reference solution (a). Add 5 ml of a 30 per cent w/v solution of *sodium iodide* in *acetone* to 5.0 ml of a 0.0001 per cent w/v solution of *busulphan IPRS* in *acetone*, stopper the flask lightly and heat in a water-bath at 50° for 90 minutes. Cool, add 10 ml of the internal standard solution, mix, add 10 ml of *water* and 20.0 ml of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.

Reference solution (b). Prepare in the same manner as reference solution (a) but using 10 ml of *acetone* in place of internal standard solution.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl),
- temperature:
  column. 140°,
  inlet port and detector at 240°,
- electron capture detector,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

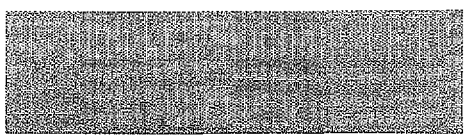
Inject 1 µl of reference solution(a), and the test solution.

Calculate the content of $C_6H_{14}O_6S_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by gas chromatography (2.4.13) as given under the test for Uniformity of content using the following test solution.

Test solution. Weigh and powder 20 tablets. Weigh a quantity of the powder containing 2.5 mg of Busulphan, add 5 ml of *water* and place in an ultrasonic bath until completely dispersed. Add 150 ml of *acetone*, shake for 15 minutes and dilute to 250.0 ml with *acetone*. Centrifuge and dilute 10.0 ml of the supernatant liquid to 100.0 ml with *acetone*. To 5.0 ml of the resulting solution add 5 ml of a 30 per cent solution of *sodium iodide* in *acetone*, stopper the flask lightly and heat in a water-bath at 50° for 90 minutes. Cool, add 10 ml of the internal standard solution, mix, add 10 ml of *water* and 20.0 ml of *hexane*, shake vigorously for 1 minute and allow to separate. Use the hexane layer.



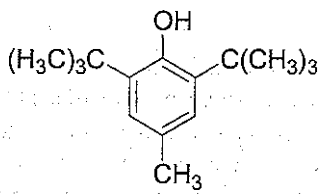
Inject 1 µl of reference solution(a), (b) and the test solution.

Calculate the content of $C_6H_{14}O_6S_2$ in the tablets.

Storage. Store protected from light.

Butylated Hydroxytoluene

BHT



$C_{15}H_{24}O$ Mol. Wt. 220.4

Butylated Hydroxytoluene is 2,6-bis(1,1-dimethylethyl)-4-methylphenol.

Category. Pharmaceutical aid (antioxidant).

Description. A white to yellowish white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *butylated hydroxytoluene IPRS* or with the reference spectrum of butylated hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *ethanol* shows an absorption maximum only at about 278 nm, between 0.40 and 0.45.

C. Dissolve about 10 mg in 2 ml of *ethanol (95 per cent)*, add 1 ml of a 0.1 per cent w/v solution of *testosterone propionate* in *ethanol (95 per cent)* and 2 ml of *2 M sodium hydroxide*, heat in a water-bath at 80° for 10 minutes and allow to cool; a blue colour is produced.

D. Dissolve about 0.1 g in 10 ml of *ethanol (95 per cent)*, add 2 ml of a 2.0 per cent w/v solution of *sodium tetraborate* and a few crystals of *2,6-dichloroquinone-4-chlorimide*; not more than a faint blue colour is produced (distinction from butylated hydroxyanisole).

E. Dissolve a few crystals in 10 ml of *ethanol (95 per cent)*, add 0.5 ml of a 0.2 per cent w/v solution of *potassium ferricyanide* and 0.5 ml of a 0.2 per cent per cent w/v solution of *ferric ammonium sulphate* in *0.5 M sulphuric acid*; a green to blue colour is produced.

Tests

Freezing point (2.4.11). 69° to 70°.

Appearance of solution. A 10.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution YS5 or BYS5 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. Dichloromethane.

Test solution. Dissolve 2.0 g of the substance under examination in 100.0 ml of *methanol.*

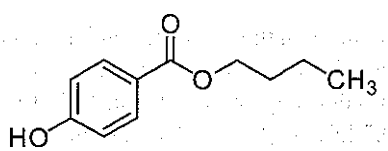
Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with a freshly prepared mixture of 70 volumes of *water*, 20 volumes of a 10.5 per cent w/v solution of *ferric chloride* and 10 volumes of *potassium ferricyanide solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Butylparaben

Butyl Hydroxybenzoate; Butyl-4-hydroxybenzoate



$C_{11}H_{14}O_3$ Mol. Wt. 194.2

Butylparaben is *n*-butyl *p*-hydroxybenzoate.

Butylparaben contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{11}H_{14}O_3$.

Category. Pharmaceutical aid.

Description. A white or almost white, crystalline powder or colourless crystals.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *butylparaben IPRS* or with the reference spectrum of butylparaben.

B. In the test for Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

C. To about 10 mg in a test-tube, add 1 ml of *sodium carbonate solution*, boil for 30 second and cool (solution A). To a further 10 mg in a similar test-tube add 1 ml of *sodium carbonate solution*; the substance partly dissolves (solution B). Add at the same time to solution A and solution B, add 5 ml of *aminopyrazolone solution* and 1 ml of *potassium ferricyanide solution*. Solution B is yellow to orange-brown. Solution A is orange to red, the color being clearly more intense than any similar color which may be obtained with solution B.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1) and not more intensely colored than reference solution BYS7 (2.4.1).

Acidity. To 2 ml of 10 per cent w/v solution in *ethanol (95 per cent)*, add 3 ml of *ethanol (95 per cent)*, 5 ml of *carbon dioxide-free water and* 0.1 ml of *bromocresol green solution*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 2.5 ml of *methanol* and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 5 mg of *4-hydroxybenzoic acid*, 5 mg of *propyl parahydroxybenzoate* (butylparaben impurity D) and 5 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 50 mg of *butyl paraben IPRS* in 2.5 ml of *methanol* and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (d). Dissolve 5 mg of *iso-butyl parahydroxybenzoate IPRS* (*butylparaben impurity E IPRS*) in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (e). Dilute 0.5 ml of reference solution (d) to 50.0 ml with reference solution (b).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 50 volumes of 0.68 per cent w/v solution of *potassium dihydrogen phosphate*, and 50 volumes of *methanol*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 10 µl.

The relative retention time with reference to butylparaben for 4-hydroxybenzoic acid is about 0.1, for butylparaben impurity D is about 0.5 and for butylparaben impurity E is about 0.9.

Inject reference solution (a) and (e). The test is not valid unless the resolution between the peaks corresponding to butylparaben and butylparaben impurity D is not less than 5.0 in the chromatogram obtained with reference solution (a) and the resolution between the peaks corresponding to butylparaben and butylparaben impurity E is not less than 1.5 in the chromatogram obtained with reference solution (e).

Inject reference solution (c) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to 4-hydroxybenzoic acid multiplied by 1.4 is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore the peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

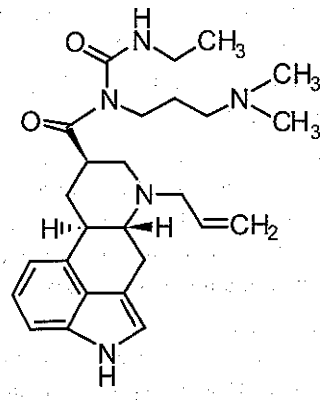
Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{11}H_{14}O_3$.

C

Cabergoline



$C_{26}H_{37}N_5O_2$ Mol. Wt. 451.6

Cabergoline is 1-[(6-Allylergolin-8β-yl)-carbonyl]-1-[3-(dimethylamino)propyl]-3-ethylurea.

Cabergoline contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{26}H_{37}N_5O_2$, calculated on the anhydrous basis.

Category. Prolactin Inhibitor.

Description. A white or almost white, crystalline powder. It shows polymorphism (2.5.11).

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cabergoline IPRS* or with the reference spectrum of cabergoline.

Tests

Specific optical rotation (2.4.22). −83° to −77°, determined in a 0.2 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and protected from light.

Test solution. Dissolve 30 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). A 0.12 per cent w/v solution of *cabergoline IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 10.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (c). Dissolve 50 mg of the substance under examination in 10.0 ml of *0.1 M sodium hydroxide*. Stir for about 15 minutes. To 1.0 ml of the suspension add 1 ml of *0.1 M hydrochloric acid* and dilute to 10.0 ml with the mobile phase. The main degradation product obtained is cabergoline impurity A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 16 volumes of *acetonitrile* and 84 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* in 1000 ml of *water* adjusted to pH 2.0 with *phosphoric acid* and 0.2 volume of *triethylamine*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time
Cabergoline impurity D[1]	0.3
Cabergoline impurity B[2]	0.6
Cabergoline impurity A[3]	0.8
Cabergoline (Retention time: about 12 minutes)	1.0
Cabergoline impurity C[4]	2.9

[1](6a*R*,9*R*,10a*R*)-*N*-[3-(dimethylamino)propyl]-7-(prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide,

[2] (6a*R*,9*R*,10a*R*)-*N*⁹-[3-(dimethylamino)propyl]-*N*⁴-ethyl- 7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline-4,9(6*H*)- dicarboxamide,

[3](6a*R*,9*R*,10a*R*)-7-(prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,

[4](6a*R*,9*R*,10a*R*)-*N*⁹-[3-(dimethylamino)propyl]-*N*⁴-ethyl-*N*⁹- (ethylcarbamoyl)-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline- 4,9(6*H*)-dicarboxamide.

Inject reference solution (c).The test is not valid unless the resolution between the peaks due to cabergoline and cabergoline impurity A is not less than 3.0.

Inject reference solution (b) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to cabergoline impurities A and C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any peak due to cabergoline impurities B and D is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent). Ignore any peak with an area less

than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{26}H_{37}N_5O_2$.

Storage. Store protected from light.

Cabergoline Tablets

Cabergoline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cabergoline, $C_{26}H_{37}N_5O_2$.

Usual strengths. 0.25 mg; 0.5 mg; 1 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2)

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. A solution of *cabergoline IPRS* in the dissolution medium suitably diluted to obtain a solution having the same concentration as that of the test solution.

Use the chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{37}N_5O_2$ in the tablets.

Q. Not less than 70 per cent of the stated amount of $C_{26}H_{37}N_5O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and protected from light.

Test solution. Disperse a quantity of the powdered tablets containing 2.5 mg of Cabergoline in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.025 per cent w/v solution of *cabergoline IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). To 10.0 ml of *0.1 M sodium hydroxide*, add 50 mg of *cabergoline IPRS*. Stir for about 15 minutes. To 1 ml of the suspension, add 1 ml of *0.1 M hydrochloric acid*, and dilute to 10.0 ml with the mobile phase. The main degradation product obtainted is cabergoline impurity A.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 16 volumes of *acetonitrile* and 84 volumes of a buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 900 ml of *water*, adjusted to pH 2.0 with *orthophosphoric acid*, dilute to 1000 ml with *water*, add 0.2 ml of *triethylamine*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 100 µl.

Name	Relative retention time
Cabergoline impurity A[1]	0.8
Cabergoline	1.0
Cabergoline impurity C[2]	1.4

[1](6a*R*,9*R*,10a*R*)-7-(Prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,

[2](6a*R*,9*R*,10a*R*)-7-Allyl-*N*-(3-(dimethylazinoyl)propyl)-*N*-(ethylcarbamoyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*] quinoline -9-carboxamide.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to cabergoline and cabergoline impurity A is not less than 3.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to cabergoline impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and the area of any peak corresponding to cabergoline impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent).

Other tests. Comply with the tests stated under Tablets.

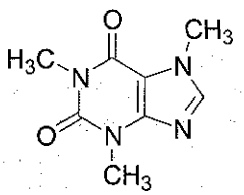
Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{26}H_{37}N_5O_2$ in the tablets.

Storage. Store protected from light and moisture.

Caffeine



$C_8H_{10}N_4O_2$ Mol. Wt. 194.2 (anhydrous)

$C_8H_{10}N_4O_2,H_2O$ Mol. Wt. 212.2 (monohydrate)

Caffeine is 3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione or its monohydrate.

Caffeine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_8H_{10}N_4O_2$, calculated on the dried basis.

Category. Central nervous system stimulant.

Description. Silky white crystals, white glistening needles or a white crystalline powder; odourless; sublimes readily.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6), after drying the substance under examination at 100° for 1 hour. Compare the spectrum with that obtained with *caffeine IPRS* or with the reference spectrum of caffeine.

B. To 10 mg in a porcelain dish, add 1 ml of *hydrochloric acid* and 0.1 g of *potassium chlorate* and evaporate to dryness on a water-bath. Expose the residue to the vapours of *dilute ammonia solution*; a purple colour is produced which disappears on addition of a solution of a fixed alkali.

C. To a saturated solution add a few drops of *tannic acid solution*; a white precipitate is produced which is soluble in excess of the reagent.

D. To 5 ml of saturated solution add 1.5 ml of *0.05 M iodine*, the solution remains clear. Add a few drops of *dilute hydrochloric acid*; a brown precipitate is formed which dissolves on neutralisation with *sodium hydroxide solution*.

Tests

Appearance of solution. A 1.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. Dissolve 0.2 g in 10 ml of boiling *water* and cool. Add 0.1 ml of *bromothymol blue solution*. The solution is coloured green or yellow. Titrate with *0.02 M sodium hydroxide* to a blue colour; not more than 0.1 ml is required.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 10 mg of the substance under examination in 10 ml of the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution. Dissolve 5 mg of *caffeine IPRS* in 5.0 ml of 0.002 per cent w/v solution of *theophylline* in the mobile phase and 10 ml of the mobile phase with the aid of ultrasound and dilute to 25.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1910 volumes of a solution prepared by dissolving 1.64 g of *anhydrous sodium acetate* in *water* and dilute to 2000 ml with *water*, 50 volumes of *acetonitrile* and 40 volumes of *tetrahydrofuran*, adjusted to pH 4.5 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 10 µl.

The relative retention time with reference to caffeine for theophylline is about 0.69.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to theophylline and caffeine is not less than 6.0 and the tailing factor for each peak is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.1 per cent and the sum of areas of all the secondary peaks is not more than 0.1 per cent, calculated by area normalization.

Arsenic (2.3.10). Mix 3.3 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). Dissolve 1.0 g in 5 ml of *0.1 M hydrochloric acid* and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent (for the anhydrous form) and between 5.0 per cent to 9.0 per cent (for the monohydrate form), determined on 1.0 g by drying in an oven at 105° for 1 hour.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *caffeine IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 955 volumes of buffer solution prepared by dissolving 0.82 g of *anhydrous sodium acetate* in 1000 ml of *water*, adjusted to pH 4.5 with *glacial acetic acid*, 25 volumes of *acetonitrile* and 20 volumes of *tetrahydrofuran*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 20 µl.

The retention time of the principal peak is about 10.0 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{10}N_4O_2$.

Storage. Store protected from light and moisture.

Labelling. The label states whether it is anhydrous or monohydrate.

Caffeine Citrate Oral Solution

Caffeine Citrate Oral Solution is a solution of caffeine citrate, prepared by the interaction of caffeine and citric acid monohydrate, in a suitable aqueous vehicle.

Caffeine Citrate Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of caffeine citrate, $C_8H_{10}N_4O_2$, $C_6H_8O_7$.

Usual strength. 20 mg per ml (equivalent to 10 mg of caffeine base).

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (a).

B. It gives reaction (B) of citrates (2.3.1).

Tests

Other tests. Comply with the tests stated under Oral Solution.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a quantity of oral solution containing 50 mg of caffeine to 250.0 ml with *water* and filter.

Reference solution (a). A 0.02 per cent w/v solution of *caffeine IPRS* in *water*.

Reference solution (b). A solution containing 0.02 per cent w/v of *caffeine IPRS* and 0.0004 per cent w/v of *theophylline* in *water*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 4 volumes of *tetrahydrofuran*, 5 volumes of *acetonitrile* and 191 volumes of *0.01M sodium acetate*, adjusted to pH 4.5 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to caffeine and theophylline is not less than 6.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_8H_{10}N_4O_2$,$C_6H_8O_7$ and determining the weight per ml (2.4.29) of the oral solution.

1 mg of $C_8H_{10}N_4O_2$ is equivalent to 0.002 g of $C_8H_{10}N_4O_2$,$C_6H_8O_7$.

Labelling. The label states the quantity of active ingredient in terms of the amount of caffeine citrate and the equivalent amount of caffeine.

Calamine

Prepared Calamine

Calamine is Zinc Oxide with a small proportion of ferric oxide.

Calamine contains not less than 98.0 per cent and not more than 100.5 per cent of ZnO, calculated on the ignited basis.

Category. Topical protectant.

Description. A fine, amorphous, impalpable, pink or reddish-brown powder.

Identification

A. Shake 1 g with 10 ml of *dilute hydrochloric acid* and filter; the filtrate gives the reactions of zinc salts (2.3.1).

B. To 1 g add 10 ml of *dilute hydrochloric acid*, heat to boiling and filter. To the filtrate add a few drops of *ammonium thiocyanate solution*; a reddish colour is produced.

Tests

Acid-insoluble substances. Not more than 1 per cent w/w, determined by the following method. Dissolve 1.0 g in 25 ml of warm *dilute hydrochloric acid*. If any insoluble residue remains, filter, wash with *water*, dry to constant weight at 105°, cool and weigh.

Alkaline substances. Digest 1.0 g with 20 ml of warm *water*, filter and add 2 drops of *phenolphthalein solution* to the filtrate. If a red colour is produced, not more than 0.2 ml of *0.05 M sulphuric acid* is required to decolorise it.

Water-soluble dyes. Shake 1.0 g with 10 ml of *water* and filter; the filtrate is colourless.

Ethanol-soluble dyes. Shake 1.0 g with 10 ml of *ethanol (90 per cent)* and filter; the filtrate is colourless.

Arsenic (2.3.10). Dissolve 1.25 g in 15 ml of *brominated hydrochloric acid AsT*, add 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (8 ppm).

Lead. Dissolve 2.0 g in a mixture of 20 ml of *water* and 5 ml of *glacial acetic acid*, filter and add 0.25 ml of *potassium chromate solution*; the solution remains clear for 5 minutes.

Calcium. Dissolve 0.5 g in a mixture of 10 ml of *water* and 2.5 ml of *glacial acetic acid* by warming on a water-bath, if necessary and filter. To 0.5 ml of the filtrate, add 15 ml of *dilute ammonia solution* and 2 ml of a 2.5 per cent w/v solution of *ammonium oxalate* and allow to stand for 2 minutes; the solution remains clear.

Soluble barium salts. To the remainder of the filtrate obtained in the test for Calcium add 2 ml of *1 M sulphuric acid* and allow to stand for 5 minutes; the solution remains clear.

Chlorides (2.3.12). Dissolve 0.33 g in *water* with the addition of 1 ml of *nitric acid* and dilute to 30 ml with *water*. The resulting solution complies with the limit test for chlorides (750 ppm).

Sulphates (2.3.17). Dissolve 0.025 g in *water* with the addition of 3 ml of *2 M hydrochloric acid* and dilute to 15 ml with *water*; filter. The filtrate complies with the limit test for sulphates (0.6 per cent).

Loss on ignition (2.4.20). Not more than 2.0 per cent, determined on 2.0 g by igniting to constant weight at a temperature not less than 900°.

Microbial contamination (2.2.9). 1.0 g is free from *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Assay. Weigh accurately about 1.5 g and digest with 50.0 ml of *0.5 M sulphuric acid*, applying gentle heat until no further solution occurs. Filter and wash the residue with hot *water* until the last washing is neutral to *litmus paper*. To the combined filtrate and washings, add 2.5 g of *ammonium chloride*, cool and titrate with *1 M sodium hydroxide* using *methyl orange solution* as indicator.

Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of *0.5 M sulphuric acid* is equivalent to 0.04068 g of ZnO.

Storage. Store protected from light and moisture.

Aqueous Calamine Cream

Calamine	40 g
Zinc Oxide	30 g
Liquid Paraffin	200 g
Self-Emulsifying Glyceryl Monostearate	50 g
Cetostearyl Alcohol	40 g
Cetomacrogol 1000	10 g
Phenoxyethanol	5 g
Purified Water, freshly boiled and cooled	625 g

Melt together the Cetostearyl Alcohol and Cetomacrogol 1000, stir until cold and dissolve this mixture and the Self-Emulsifying Glyceryl Monostearate in the Liquid Paraffin at 60°. Add with rapid stirring to a solution of the Phenoxyethanol in 450 g of the Purified Water at the same temperature and stir until cold. Triturate the Calamine and the Zinc Oxide with the remainder of the Purified Water and incorporate in the cream with stirring.

Aqueous Calamine Cream contains not less than 6.30 per cent and not more than 7.67 per cent w/w of ZnO.

Identification

The residue obtained in the Assay is yellow when hot and white when cool.

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Weigh accurately about 4.0 g. Heat carefully, taking care to avoid spurting, until the liquid is completely evaporated and the solid is charred. Ignite the residue to constant weight at a temperature of not less than 900°.

Storage. Store at a temperature not exceeding 30°. Do not freeze.

Labelling. The label states (1) the concentrations of Calamine and Zinc Oxide in the preparation; (2) that the preparation is intended for external use only; (3) the storage conditions.

Calamine Lotion

Calamine	150 g
Zinc Oxide	50 g
Bentonite	30 g
Sodium Citrate	5 g
Liquefied Phenol	5 ml
Glycerin	50 ml
Purified Water, freshly boiled and cooled sufficient to produce	1000 ml

Triturate the Calamine, the Zinc Oxide and the Bentonite with a solution of the Sodium Citrate in about 700 ml of Purified Water and add the Liquified Phenol, the Glycerin and sufficient Purified Water to produce 1000 ml. Calamine Lotion contain not less than 18.0 per cent w/v and not more 22.0 per cent w/v of zinc oxide, ZnO.

Identification

A. To 2 ml add 2 ml of *periodic acid reagent*, shake, centrifuge and add 0.5 ml of the supernatant liquid to 2 ml of *ammonical silver nitrate solution* in a test-tube; a silver mirror is produced on the walls of the tube.

B. Mix 2 ml with 50 ml of *water*, centrifuge and decant the supernatant liquid. Suspend the residue in 20 ml of *water*, add 1 ml of *hydrochloric acid*, mix and filter. 5 ml of the filtrate, after neutralisation by dropwise addition of *2 M sodium hydroxide*, gives the reactions of zinc salts (2.3.1).

Tests

Microbial contamination (2.2.9). 1.0 g is free from *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Other tests. Comply with the tests stated under Lotions.

Assay. Weigh 0.5 g in a porcelain dish, heat gently over a small flame until the base is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 ml of *2 M acetic acid* and add sufficient *water* to produce 50 ml. Keep the solution on water bath for about 10 minutes. Cool it and to the resulting solution add about 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce violet-pink colour. Add a further 2 g of *hexamine* and titrate with *0.05 M disodium edetate* until the solution becomes yellow.

Calculate the content of ZnO, determining the weight per ml (2.4.29) of the lotion.

1 ml of *0.05 M disodium edetate* is equivalent to 0.00407 g of ZnO.

Storage. Store at a temperature not exceeding 30°. Do not freeze.

Labelling. The label states (1) the concentrations of Calamine and Zinc Oxide in the preparation; (2) that the preparation is intended for external use only; (3) that the contents should be shaken before use; (4) the conditions under which the preparation should be stored.

Calamine Ointment

Calamine	150 g
White Soft Paraffin	850 g

Triturate the calamine with part of the White Soft Paraffin until smooth and gradually incorporate the remainder of the White Soft Paraffin.

Calamine Ointment contains not less than 13.5 per cent and not more than 16.5 per cent w/w of ZnO.

Identification

The residue obtained in the Assay is yellow when hot and white when cool.

Tests

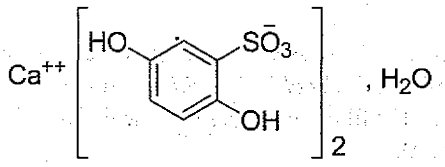
Other tests. Comply with the tests stated under Ointments.

Assay. Weigh accurately about 1.0 g. Heat gently until the base is completely volatalised or charred. Increase the heat until all the carbon is removed and ignite the residue until, after further ignition, two successive weighings do not differ by more than 0.2 per cent of the weight of the residue.

Storage. Store in well-closed containers, at a temperature not exceeding 30°.

Labelling. The label states (1) the concentration of Calamine in the preparation; (2) that the preparation is intended for external use only; (3) the storage conditions.

Calcium Dobesilate Monohydrate



$C_{12}H_{10}CaO_{10}S_2, H_2O$ Mol Wt. 436.4

Calcium Dobesilate Monohydrate is calcium di(2,5-dihydroxybenzenesulfonate) monohydrate.

Calcium Dobesilate Monohydrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{10}CaO_{10}S_2$, calculated on the anhydrous basis.

Category. Indicated in prostatic hypertrophy.

Description. A white to almost white, hygroscopic powder.

Identification

A. When examined in the range 210 nm to 350 nm (2.4.7) a 0.0025 per cent w/v solution shows absorption maxima at 221 nm and 301 nm. Specific absorbance at the absorption maximum at 301 nm is 174 to 181.

B. To 5.0 ml of solution A, add a mixture of 1 ml of *ferric chloride solution*, 1 ml of 1.0 per cent w/v solution of *potassium ferricyanide* and 0.1 ml of *nitric acid*. A blue colour and a precipitate are immediately produced.

C. 2 ml of solution A gives reaction (A) of calcium (2.3.1).

Tests

Solution A. A 10.0 per cent w/v solution in *carbon dioxide-free water*.

Appearance of solution. Solution A is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 4.5 to 6.0, determined on solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *water*. Dilute 1.0 ml of the solution to 10.0 ml with *water*.

Reference solution (b). A solution containing 0.001 per cent w/v, each of, the substance under examination and dobesilate impurity A in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with spherical end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of buffer solution prepared by mixing 1.2 g of *anhydrous sodium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH 6.5 with *disodium hydrogen phosphate solution* and dilute to 1000 ml with *water*.
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Dobesilate (Retention time: about 6 minutes)	1.0	---
Dobesilate impurity A[1]	1.7	0.6

[1]hydroquinone.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to dobesilate and dobesilate impurity A is not less than 8.0.

Inject reference solution (a) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with test solution, the area of the peak due to dobesilate impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.33 g complies with the limit test for heavy metals, method B (15 ppm).

Iron (2.3.14). 10 ml of solution A complies with the limit test for iron (10 ppm), using 1.0 ml of *iron standard solution (10 ppm)*.

Water (2.3.43). 4.0 to 6.0 per cent, determined on 0.5 g.

Assay. Dissolve 0.2 g in a mixture of 10 ml of *water* and 40 ml of *dilute sulphuric acid*. Titrate with *0.1 M cerium sulphate*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M cerium sulphate* is equivalent to 0.01045 g of $C_{12}H_{10}CaO_{10}S_2$.

Storage. Store protected from light and moisture.

Calcium Stearate

Octadecanoic acid, calcium salt

Calcium Stearate, is a compound of calcium with a mixture of solid organic acids obtained from fats and consists chiefly of variable proportions of calcium stearate and calcium palmitate.

Calcium Stearate contains the equivalent of not less than 9.0 per cent and not more than 10.5 per cent of calcium oxide (CaO). Stearic acid in the fatty acid fraction is not less than 40.0 per cent and sum of stearic acid and palmitic acid in the fatty acid fraction is not less than 90.0 per cent.

Category. Pharmaceutical aid.

Description. A white or almost white powder.

Identification

A. Heat 1 g with a mixture of 25 ml of *water* and 5 ml of *hydrochloric acid*; fatty acids are liberated and appear as an oily layer floating on the surface of the liquid. The water layer gives the tests for calcium (2.3.1).

B. Mix 25 g with 200 ml of hot *water*, add 60 ml of *2 M sulphuric acid*, and heat the mixture, with frequent stirring, until the separated fatty acid layer is clear. Wash the fatty acids with boiling *water* until free from sulphate, collect them in a small beaker, and warm on a steam bath until the *water* has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, melt the acids, filter into a dry beaker, and dry at 105° for 20 minutes; the fatty acids so obtained congeal at a temperature not below 54° (2.4.10).

Tests

Compositions of fatty acids. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.1 g of the substance under examination in 5 ml of *boron trifluoride- methanol solution*. Boil under a reflux condenser for 10 minutes. Add 4 ml of *heptane* through the condenser. Boil under a reflux condenser for 10 minutes. Allow to cool. Add 20 ml of a saturated *sodium chloride solution*. Shake and allow the layers to separate. Remove about 2 ml of the organic layer and dry over 0.2 g of *anhydrous sodium sulphate*. Dilute 1.0 ml of the solution to 10.0 ml with *heptane*.

Reference solution. Dissolve 50.0 mg, each of, *palmitic acid IPRS* and *stearic acid IPRS* in 5 ml of *boron trifluoride-methanol solution*. Boil under a reflux condenser for 10 minutes. Add 4 ml of *heptane* through the condenser. Boil under a reflux condenser for 10 minutes. Allow to cool. Add 20 ml of a saturated *sodium chloride solution*. Shake and allow the layers to separate. Remove about 2 ml of the organic layer and dry over 0.2 g of *anhydrous sodium sulphate*. Dilute 1.0 ml of the solution to 10.0 ml with *heptane*.

Chromatographic system

- a capillary column 30 m x 0.32 mm, packed with fused silica coated with macrogol 20000 (film thickness 0.5 µm);
- temperature:

column	time (min)	temperature (°)
	0-2	70
	2-36	70-240
	36-41	240

- Inlet port at 220° and detector at 260°,
- flame ionization detector,
- flow rate: 2.4 ml per minute using nitrogen as carrier gas.

The relative retention time with reference to methyl stearate for methyl palmitate is about 0.88.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks due to methyl stearate and methyl palmitate is not less than 5.0.

Inject the reference solution and the test solution.

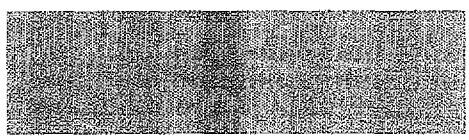
Calculate the content of palmitic acid and stearic acid.

Heavy metals (2.3.13).Place 2.5 g in a porcelain dish, place a 500 mg portion in a second dish to provide the control, and to each add 5 ml of a 1 in 4 solution of *magnesium nitrate* in *alcohol*. Cover the dishes with 7.5 cm short-stem funnels so that the stems are straight up. Heat on a hot plate at low heat for 30 minutes, then heat at medium heat for 30 minutes, and cool. Remove the funnels, add 2 ml of *standard lead solution* (*20 ppm Pb*) to the control, and heat each dish over a suitable burner until most of the carbon is burned off. Cool, add 10 ml of *nitric acid*, and transfer the solutions into 250 ml beakers. Add 5 ml of 70 per cent *perchloric acid*, cautiously evaporate to dryness, add 2 ml of *hydrochloric acid* to the residues, and wash down the insides of the beakers with *water*. Evaporate carefully to dryness again, swirling near the dry point to avoid spattering. Repeat the hydrochloric acid treatment, then cool, and dissolve the residues in about 10 ml of *water*. To each solution add 1 drop of *phenolphthalein solution* and add *sodium hydroxide solution* until the solutions just turn pink, then add *3 M hydrochloric acid* until the solutions become colourless. Add 1 ml of *1 M acetic acid* and a small amount of charcoal to each solution, and filter through filter paper into 50 ml Nessler cylinders. Wash with water, dilute with *water* to 40 ml, add 1.2 ml of *thioacetamide reagent* and 2 ml of *pH* 3.5 *acetate buffer* to each tube, and allow to stand for 5 minutes; the color of the test solution does not exceed that of the control *(10 ppm Pb)*.

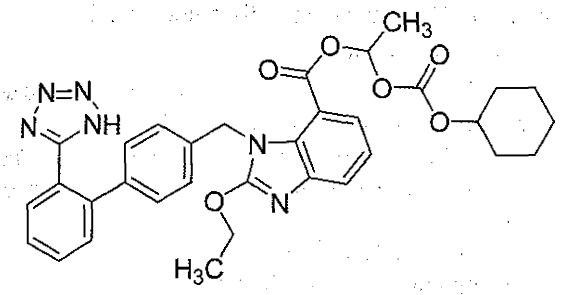
Loss on drying (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Boil about 1.2 g accurately weighed, with 50 ml of *1 M sulphuric acid* for about 3 hours using a watch glass cover to avoid splattering, or until the separated fatty acid layer is clear, adding *water*, if necessary to maintain the original volume. *[Note-Stirring may be helpful in obtaining a clear layer and decreasing extraction time.]* Cool, filter, and wash the filter and the flask thoroughly with *water* until the last washing is not acid to *litmus*. Neutralize the filtrate with 1 *M sodium hydroxide* to *litmus*. While stirring, preferably with a magnetic stirrer, titrate with *0.05 M disodium edetate* as follows. Add about 30 ml from a 50-ml burette, then add 1 ml of *1 M sodium hydroxide* and 300 mg of *hydroxy naphthol blue*, and continue the titration to a blue end-point.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002804 g of calcium oxide.



Candesartan Cilexetil



$C_{33}H_{34}N_6O_6$ Mol. Wt. 610.7

Candesartan Cilexetil is (±)-1-Hydroxyethyl 2-ethoxy-1-[p-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester).

Candesartan Cilexetil contains not less than 98.7 per cent and not more than 101.0 per cent of $C_{33}H_{34}N_6O_6$, calculated on anhydrous basis.

Category. Antihypertensive.

Production

As Nitrosamines are classified as probable human carcinogens, their presence in candesartan cilexetil should be avoided or limited as much as possible. For this reason, manufacturers of candesartan cilexetil for human use are expected to perform an assessment of the risk of *N*-nitrosamine formation and contamination during their manufacturing process; if this assessment identifies a potential risk, the manufacturing process should be modified to minimise contamination and a control strategy implemented to detect and control *N*-nitrosamine impurities in candesartan cilexetil. The general chapter 5.11. Nitrosamine Impurities is available to assist manufacturers.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *candesartan cilexetil IPRS* or with the reference spectrum of candesartan cilexetil.

B. In the Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *acetonitrile* and 40 volumes of *water*.

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.004 per cent w/v of *candesartan cilexetil IPRS* and 0.0125 per cent w/v of *acenaphthene* in the solvent mixture.

Reference solution (b). A 0.0004 per cent w/v solution of *candesartan cilexetil IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: A. a mixture of 57 volumes of *acetonitrile*, 1 volume of *glacial acetic acid* and 43 volumes of *water*.
  B. a mixture of 90 volumes of *acetonitrile* 1 volume of *glacial acetic acid* and 10 volumes of *water*.
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
30	0	100
31	100	0
40	100	0

Name	Relative retention time
Ethyl candesartan A[1]	0.4
Desethyl candesartan cilexetil B[2]	0.5
Candesartan Cilexetil	1.0
N^2-Ethyl candesartan cilexetil C[3]	2.0

[1]Ethyl 1-{[2-(1*H*-tetrazol-5-yl) biphenyl-4-yl]methyl}-2-ethoxy-benzimidazole-7-carboxylate,

[2]±1-(Cyclohexyloxycarbonyloxy)ethyl 1-{[2-(1*H* -tetrazol-5-yl) biphenyl-4-yl]methyl}-2-oxobenzimidazole-7-carboxylate.

[3]±1-(Cyclohexyloxycarbonyloxy) ethyl 2-ethoxy-1-{[2-(*N*-ethyl-tetrazol-5-yl) biphenyl-4-yl]methyl} benzimidazole-7-carboxylate.

[NOTE —The mobile phase used for testing system suitability is 100 per cent mobile phase A in an isocratic mode].

Inject reference solution (a) and (b). The test is not valid unless the resolution between candesartan cilexetil and acenaphthene is not less than 5.0 for reference solution (a) and the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 3.0 per cent for reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to ethyl candesartan and N^2 Ethyl candesartan cilexetil, each is not more than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution

(b) (0.2 per cent), the area of any peak due to desethyl candesartan cilexetil is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all secondary peaks is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.3 per cent, determined on 1 g.

Assay. Dissolve 0.85 g in a 100 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.06107 g of $C_{33}H_{34}N_6O_6$.

Storage. Store protected from moisture and at a temperature not exceeding 30°.

Candesartan Cilexetil Tablets

Candesartan Cilexetil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of candesartan cilexetil $C_{33}H_{34}N_6O_6$.

Usual strengths. 4 mg; 8 mg; 16 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. When examined in the range of 200 to 400 nm (2.4.7), a 0.08 per cent w/v solution in a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water* shows an absorption maxima and minima as that of reference solution prepared in the similar manner as test solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of 0.35 per cent w/v solution of *polysorbate 20* in *0.05 M phosphate buffer pH 6.5*.

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.045 per cent w/v solution of *candesartan cilexetil IPRS* in *acetonitrile*. Dilute 1.0 ml of the solution to 100.0 ml with the dissolution medium.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *acetonitrile*, 0.1 volume of *trifluoroacetic acid* and 45 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Q. Not less than 80 per cent of the stated amount of $C_{33}H_{34}N_6O_6$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of candesartan cilexetil with 30 ml of *acetonitrile* and ultrasound for 15 minutes with intermittent shaking in cold water and dilute to 50.0 ml with *acetonitrile* and filter.

Reference solution (a). A solution containing 0.005 per cent w/v, each of, *candesartan cilexetil impurity A IPRS*, *candesartan cilexetil impurity B IPRS*, *candesartan cilexetil impurity D IPRS* and *candesartan cilexetil impurity F IPRS* in *acetonitrile*.

Reference solution (b). A 0.01 per cent w/v solution of *candesartan cilexetil IPRS* in *acetonitrile*.

Reference solution (c). A 0.05 per cent w/v solution of *candesartan cilexetil impurity G IPRS* in *methanol*.

Reference solution (d). Dilute 3.0 ml of reference solution (a), 1.0 ml each of reference solution (b) and (c) to 100.0 ml with *acetonitrile*.

Reference solution (e). Dilute 1.0 ml of reference solution (b) to 100.0 ml with *acetonitrile*.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile*, 0.1 volume of *trifluoroacetic acid* and 90 volumes of *water*,

  B. a mixture of 90 volumes of *acetonitrile*, 0.1 volume of *trifluoroacetic acid* and 10 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
30	5	95
45	5	95
50	65	35
55	65	35

Name	Relative retention time	Correction factor
Candesartan Cilexetil impurity G[1]	0.17	0.8
Candesartan Cilexetil impurity A[2*]	0.46	0.9
Candesartan Cilexetil impurity B[3]	0.77	—
Candesartan Cilexetil	1.0	—
Candesartan Cilexetil impurity D[4]	1.15	—
Candesartan Cilexetil impurity F[5]	1.47	1.1

[1]1-{[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylic acid,

[2]Ethyl 1-{[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate,

[3]1-(Cyclohexyloxycarbonyloxy)ethyl 1-{[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-hydroxybenzimidazole-7-carboxylate,

[4]1-{[(Cyclohexyloxy)carbonyl]oxy}ethyl3-({2'-(2-ethyl-1*H*-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl}methyl)-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate,

[5]1-(Cyclohexyloxycarbonyloxy)ethyl2-ethoxy-1-{[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl}benzimidazole-7-carboxylate.6l3-*O*-decladinosylazithromycin,

*It is a Process related impurity not included in total impurities.

Inject reference solution (d) and (e). The test is not valid unless the resolution between candesartan cilexetil impurity B and candesartan cilexetil peaks is not less than 5.0 with reference solution (d), the tailing factor is not more than 2.0 and the relative standard deviation is not more than 10 per cent with reference solution (e).

Inject reference solution (e) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to candesartan cilexetil impurity G is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (e) (1.0 per cent), the area of any peak due to candesartan cilexetil impurity B and F, each is not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (e) (1.5 per cent), the area of any peak due to candesartan cilexetil impurity D is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.5 per cent). The area of any other secondary peak is not more than twice the area of the principal peak in the chromatogram obtained reference solution (e) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 40 times the area of the principal peak in the chromatogram obtained with reference solution (e) (4.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following solutions.

Test solution. Disperse 1 tablet with sufficient quantity of the solvent mixture with the aid of the ultrasound to obtain a solution containing 0.04 per cent w/v of candesartan cilexetil.

Reference solution. A 0.04 per cent w/v solution of *candesartan cilexetil IPRS* in the solvent mixture.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14),

Solvent mixture. 70 volumes of *acetonitrile* and 30 volumes of *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 20 mg of Candesartan Cilexitil in about 20 ml of the solvent mixture with the aid of ultrasound for 25 minutes with intermittent shaking. Allow to cool and dilute to 25.0 ml with the solvent mixture and filter.

Reference solution. A 0.08 per cent w/v solution of *candesartan cilexetil IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *acetonitrile*, 0.1 volume of *trifluoroacetic acid* and 45 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{33}H_{34}N_6O_6$.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Candesartan Cilexetil and Hydrochlorothiazide Tablets

Candesartan Cilexetil and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of stated amount of candesartan cilexetil, $C_{33}H_{34}N_6O_6$ and hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

Usual strength. Candesartan Cilexetil, 16 mg and Hydrochlorothiazide, 12.5 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a 0.35 per cent w/v *polysorbate 20* in 0.05 *M phosphate buffer pH 6.5*.

Speed and Time: 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14),

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). A 0.072 per cent w/v solution of *candesartan cilexetil IPRS* in *acetonitrile*.

Reference solution (b). A 0.028 per cent w/v solution of *hydrochlorothiazide IPRS* in *acetonitrile*.

Reference solution (c). Dilute suitable volume of reference solution (a) and reference solution (b) in the dissolution medium to obtain a solution having similar concentration to that of the test solution.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- sample temperature: 10°,
- mobile phase: A. a mixture of 10 volumes of *acetonitrile*, 90 volumes of *water* and 0.1 volume of *trifluoroacetic acid*,

  B. a mixture of 90 volumes of *acetonitrile*, 10 volumes of *water* and 0.1 volume of *trifluoroacetic acid*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 264 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
3	80	20
5	30	70
10	30	70
13	80	20
16	80	20

Inject reference solution (c). The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for both the peaks is not more than 2.0 per cent.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{33}H_{34}N_6O_6$ and $C_7H_8ClN_3O_4S_2$ in the medium.

Q. Not less than 80 per cent each of the stated amount of $C_{33}H_{34}N_6O_6$ and $C_7H_8ClN_3O_4S_2$.

Related substances. Determine by liquid chromatography (2.4.14),

Solvent mixture A. 70 volumes of *acetonitrile* and 30 volumes of *water*.

Solvent mixture B. 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 75 mg of Candesartan Cilexetil in 30 ml of solvent mixture A with the aid of ultrasound for 20 minutes with intermittent shaking in cold water and dilute to 50.0 ml with solvent mixture A.

Reference solution (a). A solution containing 0.005 per cent w/v, each of, *benzothiadiazine related compound A IPRS*, *hydrochlorothiazide IPRS* and 0.01 per cent w/v of *chlorothiazide IPRS* in solvent mixture B. Dilute 5.0 ml of the solution to 100.0 ml with solvent mixture A.

Reference solution (b). A solution containing 0.16 per cent w/v of *candesartan cilexetil IPRS* and 0.06 per cent w/v of *hydrochlorothiazide IPRS* in solvent mixture A. Dilute 1.0 ml of the solution to 200.0 ml with solvent mixture A.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: A. a mixture of 10 volumes of *acetonitrile*, 90 volumes of *water* and 0.1 volume of *trifluoroacetic acid*,

  B. a mixture of 90 volumes of *acetonitrile*, 10 volumes of *water* and 0.1 volume of *trifluoroacetic acid*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
8	95	5
15	60	40
20	60	40
30	40	60
35	30	70
45	20	80
50	0	100
60	0	100
62	95	5
70	95	5

Name	Relative retention time	Correction factor
Candesartan cilexetil related compound G[1]	0.51	0.90
Candesartan cilexetil related compound A[2]*	0.73	—
Benzothiadiazine related compound A[3]	0.75	0.87
Chlorothiazide[4]	0.85	2.08
Candesartan cilexetil related compound B[5]	0.89	1.11
Candesartan cilexetil	1.00	—
Candesartan cilexetil related compound D[6]	1.06	1.10
Candesartan cilexetil related compound F[7]	1.24	1.20

[1]1-{[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylic acid,

[2]Ethyl 1-{[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-2-ethoxybenzimidazole-7-carboxylate,

*Process related impurity not included in Total impurities,

[3]4-Amino-6-chloro-1,3-benzenedisulfonamide,

[4]6-Chloro-2-*H*-1,2,4-benzothiadiazine-7-sulfonamide-1-1-dioxide,

[5]1-(Cyclohexyloxycarbonyloxy)ethyl-1-{[2'-(1H-tetrazol-5-yl) biphenyl-4-yl]methyl}-2-hydroxybenzimidazole-7-carboxylate,

[6]1-{[(Cyclohexyloxycarbonyloxy)carbonyl]oxyethyl 3-{[2'-(2-ethyl-2*H*-tetrzol-5-yl)biphenyl-4-yl]methyl}-2-oxo-2,3-dihydro-1H-benzimidazole-4-carboxylate,

[7]1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-{[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl}benzimidazole-7-carboxylate.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks corresponding to benzothiadiazine related compound A and chlorothiazide is not less than 1.5, chlorothiazide and hydrochlorothiazide is not less than 1.5 in the chromatogram obtained with reference solution (a). The tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 10.0 per cent for both candesartan cilexetil and hydrochlorothiazide peaks in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to candesartan cilexetil related compound G is not more than 1.9 times the area of the candesartan cilexetil peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any peak due to benzothiadiazine related compound A is not more than 5 times the area of the hydrochlorothiazide peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any peak due to chlorothiazide is not more than 2.5 times the area of the hydrochlorothiazide peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak due to candesartan cilexetil related compound B is not more than 3.28 times the area of the candesartan cilexetil peak in the chromatogram obtained with reference solution (b) (1.75 per cent), the area of any peak due to candesartan cilexetil related compound D is not more than 0.94 times the area of the candesartan cilexetil peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak due to candesartan cilexetil related compound F is not more than 2.82 times the area of the candesartan cilexetil peak in the chromatogram obtained with reference solution (b) (1.5 per cent), the area of any other secondary peak is not more than 0.38 times the area of the candesartan cilexetil peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 7.5 times the area of the candesartan cilexetil peak in the chromatogram obtained with reference solution (b) (4.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14),

Solvent mixture. 70 volumes of *acetonitrile* and 30 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 32 mg of Candesartan Cilexetil in the solvent mixture with the aid of ultrasound for about 25 minutes with intermediate shaking and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.32 per cent w/v solution of *candesartan cilexetil IPRS* in the solvent mixture.

Reference solution (b). A 0.125 per cent w/v solution of *hydrochlorothiazide IPRS* in the solvent mixture.

Reference solution (c). Dilute a suitable volume of reference solution (a) and reference solution (b) in the solvent mixture to obtain a solution having similar concentration to that of the test solution.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile*, 90 volumes of *water* and 0.1 volume of *trifluoroacetic acid*,

  B. a mixture of 90 volumes of *acetonitrile*, 10 volumes of *water* and 0.1 volume of *trifluoroacetic acid*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
4	90	10
6	30	70
15	30	70
17	90	10
20	90	10

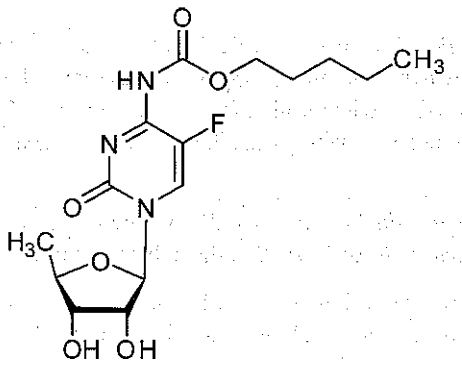
Inject reference solution (c). The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for both the components.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{33}H_{34}N_6O_6$ and $C_7H_8ClN_3O_4S_2$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Capecitabine



$C_{15}H_{22}FN_3O_6$ Mol. Wt. 359.4

Capecitabine is 5′-deoxy-5-fluoro-*N*-[(pentyloxy)carbonyl] cytidine.

Capecitabine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{22}FN_3O_6$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A white to off-white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *capecitabine IPRS* or with the reference spectrum of capecitabine.

B. In the Assay, the retention time of principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). + 96° to + 100°, determined on 1.0 per cent w/v solution in *methanol*, at 20°.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 1.0 per cent the area of the principal peak. The sum of all the secondary peaks is not more than 2.0 per cent the area of the principal peak.

Heavy metals (2.3.13). 1.0 g of complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.3 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water*, 35 volumes of *methanol* and 5 volumes of *acetonitrile*.

Test solution. Dissolve about 60 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution. A 0.06 per cent w/v solution of *capecitabine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3),
- column temperature: 40°,
- sample temperature. 5°,
- mobile phase: A. a mixture of 60 volumes of 0.1 per cent v/v solution of *acetic acid*, 35 volumes of *methanol* and 5 volumes of *acetonitrile*,
  B. a mixture of 80 volumes of *methanol*, 15 volumes of 0.1 per cent v/v solution of *acetic acid* and 5 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume:10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
20	49	51
30	49	51
31	100	0
40	100	0

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{22}FN_3O_6$.

Storage. Store protected from moisture.

Capecitabine Tablets

Capecitabine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of capecitabine, $C_{15}H_{22}FN_3O_6$.

Usual strength. 500 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve an accurately weighed quantity of *capecitabine IPRS* in the mobile phase and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.

Use chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{22}FN_3O_6$.

Q. Not less than 80 per cent of the stated amount of $C_{15}H_{22}FN_3O_6$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing about 100 mg of Capecitabine in the mobile phase, sonicate for 30 minutes and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.001 per cent w/v solution of *capecitabine IPRS* in the mobile phase.

Use chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Other tests. Comply with the tests stated in the Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and poder 20 tablets. Disperse a quantity of powder containing about 100 mg of Capecitabine in the mobile phase, sonicate for 30 minutes and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *capecitabine IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as YMC-pack),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 2.5 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 4.5 with *trifluoroacetic acid*, 20 volumes of *acetonitrile*, and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates are not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

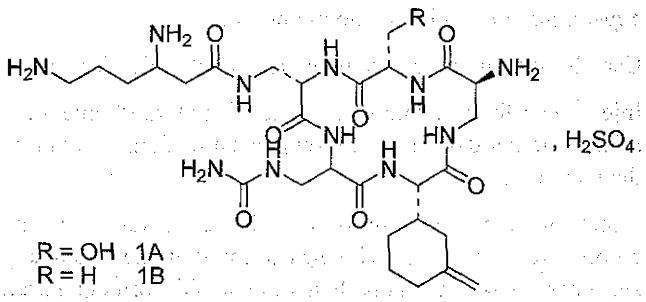
Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{22}FN_3O_6$.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the amount of Capecitabine.

Capreomycin Sulphate



Capreomycin 1A (free base)

$C_{25}H_{44}N_{14}O_8$ Mol. Wt. 668.71

Capreomycin 1A is 3,6-Diamino-*N*-({(2*S*,5*S*,11*S*,15*S*,*Z*)-15-amino-2-(hydroxymethyl)-11-[(*R*)-iminohexahydropyrimidin-4-yl]-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl}methyl)hexanamide.

Capreomycin 1B (free base)

$C_{25}H_{44}N_{14}O_7$ Mol. Wt. 652.71

Capreomycin 1B is 3,6-Diamino-*N*-({(2*S*,5*S*,11*S*,15*S*,*Z*)-15-amino-2-(hydroxymethyl)-11-[(*R*)-iminohexahydropyrimidin-4-yl]-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl}methyl)hexanamide.

Capreomycin Sulphate is the disulphate salt of capreomycin, a polypeptide mixture produced by the growth of *Streptomyces capreolus*, suitable for parenteral use.

It has a potency equivalent to not less than 700 µg and not more than 1050 µg of capreomycin per mg.

Category. Antituberculosis.

Description. A white or almost white powder.

Identification

A. In the Capreomycin 1 content, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

B. It gives reaction A of sulphates (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *water* is clear (2.4.1), when examined immediately after preparation.

pH (2.4.24). 4.5 to 7.5, determined in a 3.0 per cent w/v solution.

Capreomycin I content. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25.0 mg of the substance under examination in 100.0 ml of *water*.

Reference solution. A 0.025 per cent w/v solution of *capreomycin sulphate IPRS* in *water*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) (Such as Spherisorb CN),
- mobile phase: 55 volumes of the solution prepared by dissolving 0.5 g of *ammonium bisulphate* in 1000 ml of *water*, filter and 45 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume: 20 µl.

The relative retention time with reference to capreomycin 1B for capreomycin 1A is 0.85.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is at least 1.5 and the tailing factor is not more than 3.5 for both the peaks.

In the chromatogram obtained with the test solution, the sum of the areas of the two principal peaks, due to capreomycins 1A and 1B, is not less than 90 per cent of the total areas of all the peaks.

Sulphated ash (2.3.18). Not more than 3.0 per cent.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 0.1 g by drying in an oven for 4 hours at 100° at a pressure not exceeding 0.7 kPa.

Assay. Determine by the microbiological assay of antibiotics (2.2.10).

Caproemycin Sulphate intended for use in the manufacture of parenteral preparations complies with the following additional requirements.

Bacterial endotoxins (2.2.3). Not more than 0.35 Endotoxin Unit per mg of capreomycin.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture.

Capreomycin Injection

Capreomycin Injection is a sterile material consisting of Capreomycin Sulphate with or without auxiliary agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Usual strength. 1 g.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Capreomycin injection contains an amount of Capreomycin Sulphate equivalent to not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of capreomycin.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. In the Capreomycin 1 content, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

B. It gives reaction A of sulphates (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *water* is clear (2.4.1), when examined immediately after preparation.

pH (2.4.24). 4.5 to 7.5, determined in a 3.0 per cent w/v solution.

Capreomycin I content. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the injection containing about 25 mg of capreomycin in 100 ml of *water*.

Reference solution. A 0.025 per cent w/v solution of *capreomycin sulphate IPRS* in *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) (Such as Spherisorb CN),
- mobile phase: a mixture of 55 volumes of a solution prepared by dissolving 0.5 g of *ammonium bisulphate* in 1000 ml of *water*, filtered and 45 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume: 20 µl.

he relative retention time with reference to capreomycin 1B for capreomycin 1A is 0.85.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is at least 1.5 and the tailing factor is not more than 3.5 for both the peaks.

In the chromatogram obtained with the test solution, the sum of the areas of the two principal peaks, due to capreomycins 1A and 1B, is not less than 90 per cent of the total areas of all the peaks.

Bacterial endotoxins (2.2.3). Not more than 0.35 Endotoxin Unit per mg of capreomycin.

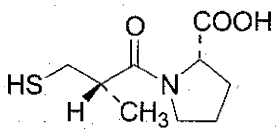
Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 0.1 g by drying in an oven at 100°at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Determine by the microbiological assay of antibiotics (2.2.10).

Storage. Store protected from moisture, at a temperature not exceeding 25°.

Labelling. The label states the quantity of Capreomycin Sulphate in terms of the equivalent amount of capreomycin.

Captopril



$C_9H_{15}NO_3S$ Mol. Wt. 217.3

Captopril is 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline.

Captopril contains not less than 97.5 per cent and not more than 102.0 per cent of $C_9H_{15}NO_3S$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *captopril IPRS* or with the reference spectrum of captopril.

B. Complies with the test for Specific optical rotation.

C. Melting range (2.4.21) 104° to 110°.

Tests

Specific optical rotation (2.4.22). – 134° to – 125°, determined in a 1.0 per cent w/v solution in *ethanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50.0 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 10.0 mg of the substance under examination in the mobile phase, add 0.25 ml of *0.05 M iodine* and dilute to 100.0 ml with the mobile phase. Dilute 10 ml of the solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.0 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.05 volume of *orthophosphoric acid*, 50 volumes of *methanol* and 50 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (b). The chromatogram shows three peaks. The test is not valid unless the resolution between the last 2 eluting peaks is not less than 2.0.

Inject reference solution (a) and the test solution. Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with a retention time less than 1.4 minutes.

Heavy metals (2.3.13). 0.66 g complies with the limit test for heavy metals, Method B (30 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Weigh 0.3 g, dissolve in 100 ml of *water* in a stoppered-flask, add 10 ml of *1.8 M sulphuric acid* and 1 g of *potassium iodide*. Titrate with *0.025 M potassium iodate* using 3 ml of *starch solution*, added towards the end-point, as indicator.

1 ml of *0.025 M potassium iodate* is equivalent to 0.03259 g of $C_9H_{15}NO_3S$.

Storage. Store protected from moisture.

Captopril Tablets

Captopril Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of captopril, $C_9H_{15}NO_3S$.

Usual strengths. 12.5 mg; 25 mg; 50 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary, at the maximum at about 212 nm.

Calculate the content of $C_9H_{15}NO_3S$ in the medium from the absorbance obtained from a solution of known concentration of *captopril IPRS*.

Q. Not less than 80 per cent of the stated amount of $C_9H_{15}NO_3S$.

Captopril disulphide. Determine by liquid chromatography (2.4.14)

Test solution. Transfer a quantity of the powdered tablets containing 25 mg of Captopril to a centrifuge tube, add 25 ml of *methanol*, centrifuge for 15 minutes and use the supernatant liquid.

Reference solution (a). A 0.003 per cent w/v solution of *captopril disulphide IPRS* in *methanol*.

Reference solution (b). Dilute 1.0 ml of test solution to 100.0 ml with reference solution (a).

Chromatographic system.
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.5 volume of *orthophosphoric acid*, 45 volumes of *water* and 55 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution (b). The test is not valid unless, the resolution between the peaks due to captopril and captopril disulphide is not less than 2.0.

Inject reference solution (a)and the test solution. In the chromatogram obtained with test solution the area of any peak corresponding to captopril disulphide is not more than the area of the peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from exposure to air and use within 8 hours of preparation.

Test solution. Disperse a quantity of the powdered tablets containing about 25.0 mg of Captopril in 25.0 ml of the mobile phase with the aid of ultrasound for 15 minutes, centrifuge and use the clear supernatant liquid.

Reference solution. A 0.1 per cent w/v solution of *captopril IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 55 volumes of *methanol* and 45 volumes of *water* containing 0.05 volumes of *phosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_{15}NO_3S$ in the tablets.

Storage. Store protected from moisture.

Captopril and Hydrochlorothiazide Tablets

Captopril and Hydrochlorothiazide tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of captopril $C_9H_{15}NO_3S$ and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

Usual strengths. Captopril, 25 mg and Hydrochlorothiazide, 15 mg; Captopril, 50 mg and Hydrochlorothiazide, 25 mg; Captopril, 25 mg and Hydrochlorothiazide, 25 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of *0.1 M hydrochloric acid,*

Speed and time. 50 rpm and 20 minutes for captopril and 30 minutes for hydrochlorothiazide,.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution(a). A 0.028 per cent w/v solution of *captopril IPRS* in dissolution medium.

Reference solution(b). A 0.028 per cent w/v solution of *hydrochlorothizide IPRS* in dissolution medium.

Reference solution(c). Dilute reference solution (a) and (b) with the dissolution medium to obtain a solution having a known concentration similar to the test solution.

Use the chromatographic system as described under Assay.

Inject reference solution (c). The relative standard deviation for replicate injections for each peak is not more than 3.0 per cent.

Inject reference solution (c) and the test solution.

Q. Not less than 80 per cent of the stated amount of captopril $C_9H_{15}NO_3S$ and not less than 60 per cent of the stated amount of hydrochlorothiazide $C_7H_8ClN_3O_4S_2$.

Captopril disulphide. Determine by liquid chromatography (2.4.14). Not more than 3.0 per cent.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of captopril in 50-ml volumetric flask, add 20 ml of the mobile phase and sonicate for 15 minutes with occasional shaking. Dilute with the mobile phase to volume, and centrifuge. Use the clear supernatant.

Reference solution (a). A solution containing 0.00075 per cent w/v, each of, *Captopril IPRS, hydrochlorothiazide IPRS* and 0.0015 per cent w/v of *Captopril disulphide IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0015 per cent w/v of *Captopril disulphide IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 550 volumes of *water*, 450 volumes of *methanol* and 0.5 volumes of *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

The relative retention time with reference to captopril disulphide for captopril is about 0.3.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to captopril and captopril disulphide is not less than 4.0 obtained with reference solution (a). Both peaks are properly resolved from hydrochlorothiazide peak and the relative standard deviation for replicate injections is not more than 3.0 per cent obtained with reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of captopril disulphide.

Benzothiadiazine related impurity A. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Reference solution. A solution containing 0.001 per cent w/v of *benzothiadiazine impurity A IPRS* in the mobile phase.

Inject reference solution and test solution. In the chromatogram obtained with the test solution, the area of any peak due to Benzothiadiazine related impurity A is not more than 0.3 times the area of principal peak in the reference solution (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Transfer a quantity of the powder containing 15 mg of hydrochlorothiazide in a 50-ml volumetric flask, add about 30 ml of mobile phase. Sonicate for 15 minute with occasional shaking, dilute to volume with the mobile phase and centrifuge.

Reference solution (a). A solution containing 0.03 per cent w/v, each of, *captopril IPRS, hydrochlorothiazide IPRS* and *benzothiadiazine impurity A IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.03 per cent w/v *hydrochlorothiazide IPRS* and appropriate concentration of *captopril IPRS* in mobile phase to produce concentration similar to the test solution concentration in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 4.6 mm packed with phenyl group bonded to porous silica (5 µm),
- mobile phase: a mixture of 750 volumes of *water*, 250 volumes of *methanol* and 0.5 volumes of *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

The relative retention time for benzothiadiazine impurity A is about 0.4, for hydrochlorothiazide is about 0.5 and for captopril is about 1.0.

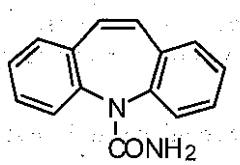
Inject reference solution (a) and (b). The test is not valid unless the resolution between the void volume and benzothiadiazine impurity A is not less than 1.7, between the benzothiadiazine impurity A and hydrochlorothiazide is not less than 1.8 and between captopril and hydrochlorothiazide is not less than 2.0 obtained with reference solution (a) and the relative standard deviation for replicate injections is not more than 3.0 per cent obtained with reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_9H_{15}NO_3S$ and $C_7H_8ClN_3O_4S_2$ in the tablets.

Storage. Store protected from moisture.

Carbamazepine



$C_{15}H_{12}N_2O$ Mol. Wt. 236.3

Carbamazepine is 5*H*-dibenz[*b*,*f*]azepine-5-carboxamide.

Carbamazepine contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{15}H_{12}N_2O$, calculated on the dried basis.

Category. Anticonvulsant.

Description. A white or yellowish-white, crystalline powder; almost odourless; exhibits polymorphism.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbamazepine IPRS* or with the reference spectrum of carbamazepine.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution (b).

Tests

Acidity or alkalinity. Stir 1.0 g with 20 ml of *carbon dioxide-free water* for 15 minutes and filter. Titrate 10 ml of the filtrate with *0.01 M sodium hydroxide* using 0.05 ml of *phenolphthalein solution* as indicator; not more than 0.5 ml is required. Add 0.15 ml of a 0.05 per cent w/v solution of *methyl red* and titrate with *0.01 M hydrochloric acid* until the colour changes to red; not more than 1.0 ml is required.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 0.15 g of the substance under examination in *methanol* and dilute to 50.0 ml with the same solvent. Mix with the aid of ultrasound and dilute 10.0 ml of the solution to 20.0 ml with *water*.

Test solution (b). Dilute 10.0 ml of test solution (a) to 50.0 ml with a mixture of equal volumes of *methanol* and *water*.

Reference solution (a). Dissolve 7.5 mg of *carbamazepine IPRS*, 7.5 mg of *10,11-dihydrocarbamazepine IPRS* and 7.5 mg of *iminodibenzyl* in *methanol* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 50.0 ml with a mixture of equal volumes of *methanol* and *water*.

Reference solution (b). Dissolve 0.15 g of *carbamazepine IPRS* in *methanol* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with a mixture of equal volumes of *methanol* and *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm),
- mobile phase: a mixture of 3 volumes of *tetrahydrofuran*, 12 volumes of *methanol* and 85 volumes of *water* adding 0.2 ml of *formic acid* and 0.5 ml of *triethylamine* to 1000 ml of the solution,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to carbamazepine and 10,11-dihydrocarbamazepine is more than 1.7.

Inject test solution (a). Run the chromatograms 6 times the retention time of carbamazepine (about 10 minutes). The area of any peak corresponding to 10,11-dihydrocarbamazepine and iminodibenzyl is not more than the areas of the corresponding peaks in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak due to carbamazepine (0.1 per cent) and the sum of all the secondary peaks is not more than 5 times the area of the principal peak due to carbamazepine (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Chlorides (2.3.12). Boil 1.5 g in 30 ml of *water* for 5 minutes, cool and filter. The filtrate complies with the limit test for chlorides (165 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using test solution (b) and reference solution (b).

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and test solution (b).

Calculate the content of $C_{15}H_{12}N_2O$.

Storage. Store protected from moisture.

Carbamazepine Prolonged-release Tablets

Carbamazepine Sustained-release Tablets;
Carbamazepine Extended-release Tablets

Carbamazepine Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Carbamazepine Prolonged-release Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of carbamazepine, $C_{15}H_{12}N_2O$.

Usual strengths. 100 mg; 200 mg; 400 mg.

Identification

Boil a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 15 ml of *acetone*, filter the hot solution, wash the filtrate with two quantities, each of 5 ml, of hot *acetone*, cool in ice, evaporate the combined filtrates to dryness. The residue complies with the following test

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbamazepine IPRS* or with the reference spectrum of carbamazepine.

Tests

Dissolution (2.5.2). Complies with the test stated under tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 ml of *methanol*, with the aid of ultrasound for 15 minutes and dilute to 200.0 ml with *water*, mix and filter.

Reference solution. Dissolve 7.5 mg, each of, *carbamazepine IPRS*, *10,11-dihydrocarbamazepine* and *iminodibenzyl* in *methanol* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the resulting solution to 50.0 ml with *methanol (50 per cent)*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm) (Such as Nucleosil 10 CN),
- mobile phase: a mixture of 30 volumes of *tetrahydrofuran*, 120 volumes of *methanol* and 850 volumes of *water*, to which is added 0.2 ml of *anhydrous formic acid* and 0.5 ml of *triethylamine*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to carbamazepine and 10, 11-dihydrocarbamazepine is at least 1.7.

Inject the reference solution and the test solution and continue the chromatography for 6 times the retention time of carbamazepine (about 10 minutes). In the chromatogram obtained with the test solution, the areas of any peaks corresponding to 10,11-dihydrocarbamazepine and iminodibenzyl are not greater than the areas of the corresponding peaks in the chromatogram obtained with the reference solution (0.1 per cent), the area of any other secondary peak is not greater than the area of the principal peak due to carbamazepine (0.1 per cent) and the sum of the areas of any such peaks is not greater than 5 times the area of the principal peak due to carbamazepine (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the peak due to carbamazepine in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.3 g of Carbamazepine with 100.0 ml of *methanol* for 15 minutes. Dilute to 200.0 ml with *water*, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with *methanol (50 per cent)*.

Reference solution. A 0.03 per cent w/v solution of *carbamazepine IPRS* in *methanol (50 per cent)*.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{12}N_2O$ in the tablets.

Storage. Store protected from moisture.

Carbamazepine Tablets

Carbamazepine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of carbamazepine, $C_{15}H_{12}N_2O$.

Usual strength. 200 mg.

Identification

Boil a quantity of the powdered tablets containing 0.2 g of Carbamazepine with 15 ml of *acetone*, filter the hot solution, wash the filtrate with two 5 ml quantities of hot acetone, cool in ice, evaporate the combined filtrates to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbamazepine IPRS* or with the reference spectrum of carbamazepine.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium: 900 ml of *water* containing 1 per cent w/v of *sodium lauryl sulphate*,
Speed and time: 75 rpm for 60 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium, if necessary. Measure the absorbance of the resulting solution at the maximum at about 288 nm (2.4.7). Calculate the content of carbamazepine, $C_{15}H_{12}N_2O$ in the medium from the absorbance obtained from a solution of known concentration of *carbamazepine IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{15}H_{12}N_2O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.3 g of Carbamazepine with 100 ml of *methanol* for 15 minutes. Dilute to 200 ml with *water*, mix and filter.

Reference solution. Dissolve 7.5 mg, each of, *carbamazepine IPRS*, *10,11-dihydrocarbamazepine* and *iminodibenzyl* in *methanol* and dilute to 100 ml with the same solvent. Dilute 1.0 ml of the resulting solution to 50.0 ml with *methanol (50 per cent)*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm) (Such as Nucleosil 10 CN),
- mobile phase: a mixture of 30 volumes of *tetrahydrofuran*, 120 volumes of *methanol* and 850 volumes of *water*, adding 0.2 ml of *anhydrous formic acid* and 0.5 ml of *triethylamine* to 1000 ml of the solution,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to carbamazepine and 10,11-dihydrocarbamazepine is at least 1.7.

Inject the test solution and continue the chromatography for 6 times the retention time of carbamazepine which is about 10 minutes.

In the chromatogram obtained with the test solution, the areas of any peaks corresponding to 10,11-dihydrocarbamazepine and iminodibenzyl are not greater than the areas of the corresponding peaks in the chromatogram obtained with the reference solution (0.1 per cent). The area of any other secondary peak is not greater than the area of the principal peak due to carbamazepine (0.1 per cent) and the sum of the areas of any such peaks is not greater than 5 times the area of the principal peak due to carbamazepine (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the peak due to carbamazepine in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.3 g of Carbamazepine with 100.0 ml of *methanol* for 15 minutes. Dilute to 200.0 ml with water, mix, filter and further dilute 1 volume of the filtrate to 5 volumes with *methanol (50 per cent)*.

Reference solution. A 0.03 per cent w/v solution of *carbamazepine IPRS* in *methanol (50 per cent)*.

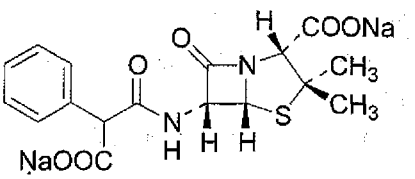
Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{12}N_2O$ in the tablets.

Storage. Store protected from moisture.

Carbenicillin Sodium

Carbenicillin Disodium



$C_{17}H_{16}N_2Na_2O_6S$ Mol. Wt. 422.4

Carbenicillin Sodium is the disodium (6*R*)-6-[(2*RS*)-2-carboxylato-2-phenylacetamido]penicillinate.

Carbenicillin Sodium contains the equivalent of not less than 770 µg of carbenicillin per mg, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or slightly yellowish powder; odourless; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbenicillin sodium IPRS* or with the reference spectrum of carbenicillin sodium.

B. Heat 0.5 g in a small sealed container on a water-bath for 3 minutes, remove the seal, and immediately replace by a cork fitted with a platinum loop carrying a drop of a solution freshly prepared by mixing 1 ml of a 0.5 per cent w/v solution of *sodium carbonate*, 1 ml of *phenolphthalein solution* and 10 ml of *water*; the reagent is decolourised within 2 minutes.

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 6.5 to 8.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +182° to +196°, determined at 20° in a 1.0 per cent w/v solution.

Iodine-absorbing substances. Not more than 8.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 0.13 g and dissolve in sufficient mixed *phosphate buffer pH 7.0* to produce 25.0 ml. To 10.0 ml add 10 ml of *mixed phosphate buffer pH 4.0* and 10.0 ml of *0.01 M iodine* and titrate immediately with *0.01 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titration represents the amount of iodine-absorbing substances present.

1 ml of *0.01 M sodium thiosulphate* is equivalent to 0.000489 g of iodine-absorbing substances.

Bacterial endotoxins (2.2.3). Not more than 0.05 Endotoxin Unit per mg of carbenicillin.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) and express the result in µg of carbenicillin per mg.

Storage. Store in sterile containers, sealed so as to exclude micro-organisms, in a refrigerator (2° to 8°).

Carbenicillin Sodium Injection

Carbenicillin Injection; Carbenicillin Disodium Injection

Carbenicillin Sodium Injection is a sterile material consisting of Carbenicillin Sodium, with or without auxilliary substances. It is filled in sealed containers.

The injection is constituted by dissolving the contents of a sealed container in the requisite amount of Water for Injections immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Carbenicillin Sodium Injection contains the equivalent of not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carbenicillin, $C_{17}H_{18}N_2O_6S$.

Usual strengths. The equivalent of 1 g and 5 g of carbenicillin.

Description. A white or almost white powder; odourless; hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbenicillin sodium IPRS* or with the reference spectrum of carbenicillin sodium.

B. Heat 0.5 g in a small sealed container on a water-bath for 3 minutes, remove the seal, and immediately replace by a cork fitted with a platinum loop carrying a drop of a solution freshly prepared by mixing 1 ml of a 0.5 per cent w/v solution of *sodium carbonate*, 1 ml of *phenolphthalein solution* and 10 ml of *water*; the reagent is decolourised within 2 minutes.

C. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 6.5 to 8.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +182° to +196°, determined at 20° in a 1.0 per cent w/v solution.

Iodine-absorbing substances. Not more than 8.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 0.13 g and dissolve in sufficient mixed *phosphate buffer pH 7.0* to produce 25.0 ml. To 10.0 ml add 10 ml of *mixed phosphate buffer pH 4.0* and 10.0 ml of *0.01 M iodine* and titrate immediately with *0.01 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination. The difference between the titration represents the amount of iodine-absorbing substances present.

1 ml of *0.01 M sodium thiosulphate* is equivalent to 0.000489 g of iodine-absorbing substances.

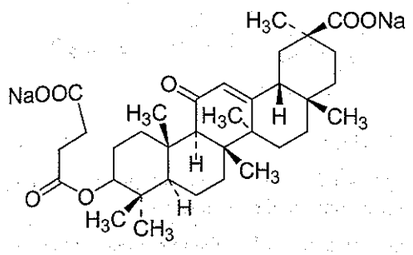
Bacterial endotoxins (2.2.3). Not more than 0.05 Endotoxin Unit per mg of carbenicillin.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

Assay. Mix the contents of 10 containers and determine by the microbiological assay of antibiotics, Method A (2.2.10) using the mixed contents of the 10 containers.

Labelling. The label states the quantity of Carbenicillin Sodium contained in the sealed container in terms of the equivalent amount of carbenicillin.

Carbenoxolone Sodium



$C_{34}H_{48}Na_2O_7$ Mol. Wt. 614.7

Carbenoxolone Sodium is disodium 3β-(3-carboxylatopropionyloxy)-11-oxoolean-12-en-30-oate.

Carbenoxolone Sodium contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{34}H_{48}Na_2O_7$, calculated on the anhydrous basis.

Category. Antiulcer.

Description. A white or pale cream powder; hygroscopic; irritant to nasal membranes.

Identification

A. Dissolve 0.1 g in 5 ml of *water*, just acidify with *2 M hydrochloric acid*, stir well and filter. Wash the residue with *water* until the washings are no longer acidic and dry to constant weight at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbenoxolone sodium IPRS* treated in the same manner or with the reference spectrum of carbenoxolone.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0025 per cent w/v solution in a mixture of equal volumes of methanol and *0.02 M sodium carbonate* shows an absorption maximum only at about 256 nm, about 0.5.

C. Mix 5 mg with 50 mg of resorcinol and 2 ml of *sulphuric acid (80 per cent)*. Heat at 200° for 10 minutes, cool, pour into 200 ml of water and add sufficient *5 M sodium hydroxide* to make the mixture just alkaline; an intense green fluorescence is produced.

D. A 5.0 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 8.0 to 9.2, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +132° to +140°, determined in a 1.0 per cent w/v solution in a mixture of equal volumes of *methanol* and *0.02 M sodium carbonate*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254* (such as precoated Merck silica gel 60 F254 plates).

Mobile phase. A mixture of 60 volumes of *ethyl acetate*, 20 volumes of *methanol*, 11 volumes of *water* and 1 volume of *strong ammonia solution*.

Test solution. A 1.5 per cent w/v solution of the substance under examination in *methanol*.

Reference solution. A 0.03 per cent w/v solution of the substance under examination in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Spray with a 1.5 per cent w/v solution of *vanillin* in *sulphuric acid (60 per cent)* and heat at 105° for 10 to 15 minutes. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.6 g.

Assay. Weigh accurately about 1.0 g and dissolve in 30 ml of *water*. Add 30 ml of *chloroform* and 15 ml of a mixture of 10 volumes of *2 M hydrochloric acid* and 90 volumes of *water*, shake and allow to separate. Add the *chloroform layer* to 40 ml of a 20 per cent w/v solution of *sodium chloride*, shake and allow to separate. Repeat the extraction with four quantities, each of 15 ml, of *chloroform*, combine the chloroform extracts and add sufficient *chloroform* to produce 100.0 ml. Evaporate 25.0 ml, dry the residue at 100° at a pressure of 2 kPa and dissolve in 10 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide* using *thymol blue solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03073 g of $C_{34}H_{48}Na_2O_7$.

Carbenoxolone Tablets

Carbenoxolone Sodium Tablets

Carbenoxolone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of carbenoxolone sodium, $C_{34}H_{48}Na_2O_7$.

Usual strength. 50 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.2 g of Carbenoxolone Sodium with 10 ml of *methanol*, filter and evaporate to dryness. The residue complies with the following tests.

1. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0025 per cent w/v solution in a mixture of equal volumes of *methanol* and *0.02 M sodium carbonate* shows an absorption maximum only at about 256 nm, about 0.5.

2. Mix 5 mg with 50 mg of *resorcinol* and 2 ml of *sulphuric acid (80 per cent)*. Heat at 200° for 10 minutes, cool, pour into 200 ml of *water* and add sufficient *5 M sodium hydroxide* to make the mixture just alkaline; an intense green fluorescence is produced.

B. A 5 per cent w/v solution of the residue obtained in test A gives the reactions of sodium salts (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 60 volumes of *ethyl acetate*, 20 volumes of *methanol*, 11 volumes of *water* and 1 volume of *strong ammonia* solution.

Test solution. Triturate a quantity of the powdered tablets containing 0.1 g of Carbenoxolone Sodium with 20 ml of *methanol*, filter, evaporate the filtrate to low volume and add sufficient *methanol* to produce 10 ml.

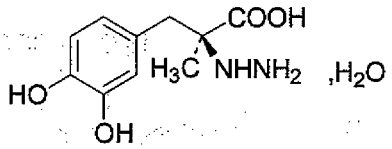
Reference solution. Dilute 3.0 ml of the test solution to 100.0 ml with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Spray with a 1.5 per cent w/v solution of *vanillin* in *sulphuric acid (60 per cent)* and heat at 105° for 10 to 15 minutes. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 75 mg of Carbenoxolone Sodium with a small volume of *methanol*, filter and add sufficient *methanol* to produce 250.0 ml. To 10.0 ml add 10 ml of *0.02 M sodium carbonate* and sufficient of a mixture of equal volumes of *methanol* and *0.02 M sodium carbonate* to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 256 nm (2.4.7). Calculate the content of $C_{34}H_{48}Na_2O_7$ taking 199 as the specific absorbance at the maximum at about 256 nm.

Carbidopa



$C_{10}H_{14}N_2O_4,H_2O$ Mol. Wt. 244.2

Carbidopa is (*S*)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid monohydrate.

Carbidopa contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{14}N_2O_4$, calculated with on the dried basis.

Category. Antiparkinson.

Description. A white to creamy white powder.

Identification

Tests A and C may be omitted if tests B, D and E are carried out. Tests B, D and E may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbidopa IPRS* or with the reference spectrum of carbidopa.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in a 1 per cent v/v solution of *hydrochloric acid* in *methanol* shows an absorption maximum only at about 282 nm, about 0.52.

C. Complies with the test for Specific optical rotation.

D. Shake vigorously about 5 mg with 10 ml of *water* for 1 minute and add 0.3 ml of *ferric chloride solution*; an intense green colour is produced, which quickly becomes reddish brown.

E. Suspend 20 mg in 5 ml of *water* and add 5 ml of *cupri-tartaric solution* and heat; the colour of the solution changes to dark brown and a red precipitate is produced.

Tests

Appearance of solution. Dissolve 0.25 g in 25 ml of *1 M hydrochloric acid*. The solution is clear (2.4.1) and not more intensely coloured than reference solution BYS6 or BS6 (2.4.1).

Specific optical rotation (2.4.22). −26.5° to −22.5°, determined in a solution prepared by dissolving 0.25 g in 25 ml of *aluminium chloride solution*.

Hydrazine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel G*.

Mobile phase. A mixture of 2 volumes of *methanol* and 1 volume of *water*.

Test solution. Place 25 g of *strongly basic anion exchange resin* into each of two stoppered conical flasks, add 150 ml of *carbon dioxide-free water* to each flask and allow to stand for 30 minutes shaking occasionally. Decant the liquid from both flasks and repeat the process with further quantities, each of 150 ml, of *carbon dioxide-free water*. Separately transfer the resin portions into two 100-ml measuring cylinders, 3.5 to 4.5 cm in internal diameter, using 60 ml of *carbon dioxide-free water* for one portion (A) and 20 ml of *carbon dioxide-free water* for the other portion (B). Into each cylinder, insert a gas-inlet tube, 2 to 3 mm in internal diameter at the end and reaching almost to the bottom of the cylinder, and pass a rapid current of *nitrogen for chromatography* through each mixture so that homogeneous suspensions are produced. After 30 minutes, without interrupting the gas flow, add 1 ml of a solution prepared by dissolving 0.5 g of the substance under examination in sufficient *2 M hydrochloric acid* to produce 2 ml to cylinder A. After 1 minute stop the gas flow to cylinder A and transfer the contents, through a moistened filter paper, into cylinder B. After 1 minute, stop the gas flow to cylinder B and immediately pour the solution through a moistened filter paper into a freshly prepared mixture of 1 ml of a 20 per cent w/v solution of *salicylaldehyde* in *methanol* and 20 ml of *phosphate buffer solution pH 5.5*, shake thoroughly for 1 minute and heat in a water-bath at 60° for 15 minutes; the liquid becomes clear. Allow to cool, add 2 ml of *toluene*, shake vigorously for 2 minutes and centrifuge. Vigorously shake the toluene layer with two quantities, each of 20 ml, of a 20 per cent w/v solution of *sodium metabisulphite* and then with two quantities, each of 50 ml, of *water* and use the toluene layer.

Reference solution. Prepare at the same time and in the same manner but using 1 ml of a 0.002 per cent w/v solution of *hydrazine sulphate* in *2 M hydrochloric acid* in place of 1 ml of the solution of the substance under examination.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution showing a yellow fluorescence is not more intense than the corresponding spot in the chromatogram obtained with the reference solution.

Methyldopa and 3-*O*-methylcarbidopa. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g in sufficient *0.1 M hydrochloric acid* to produce 10 ml.

Reference solution (a). A 0.005 per cent w/v solution, each of, *methyldopa IPR* and *3-O-methylcarbidopa IPRS* in *0.1 M hydrochloric acid*.

Reference solution (b). A 0.05 per cent w/v solution, each of, *carbidopa IPRS* and *methyldopa IPRS* in *0.1 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 98 volumes of a 1.4 per cent w/v solution of *potassium dihydrogen phosphate* and 2 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to methyldopa and carbidopa is greater than 4.0.

Inject reference solution (a) and the test solution

In the chromatogram obtained with the test solution, the areas of any peaks corresponding to methyldopa and 3-O-methylcarbidopa are not greater than the areas of the corresponding peaks in the chromatogram obtained with reference solution (a).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 6.9 to 7.9 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g and dissolve in 75 ml of *anhydrous glacial acetic acid* with the aid of gentle heat. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02262 g of $C_{10}H_{14}N_2O_4$.

Storage. Store protected from light.

Carbidopa and Levodopa Orally Disintegrating Tablets

Carbidopa and Levodopa Orally Disintegrating Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of carbidopa, $C_{10}H_{14}N_2O_4$ and levodopa, $C_9H_{11}NO_4$.

Usual strengths. 25 mg carbidopa and 100 mg levodopa; 25 mg carbidopa and 250 mg levodopa; 10 mg carbidopa and 100 mg levodopa.

Identification

In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Disintegration (2.5.1). Not more than 60 seconds.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 750 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 10 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve the suitable quantities of *carbidopa IPRS* and *levodopa IPRS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentrations similar to the expected concentrations of carbidopa and levodopa in the test solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 4°,
- mobile phase: a buffer solution prepared by dissolving 11 g of *monobasic potassium phosphate monohydrate* in 1000 ml of *water*, add 1.3 ml of 0.024 per cent w/v solution of *sodium 1-decanesulphonate* in *water*, adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

The relative retention time with reference to carbidopa for levodopa is 0.4.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{14}N_2O_4$ and $C_9H_{11}NO_4$ in the medium.

Q. Not less than 75.0 per cent of the stated amount of $C_{10}H_{14}N_2O_4$ and $C_9H_{11}NO_4$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light and use at or below 8°.

Solvent mixture. 30 volumes of *methanol* and 70 volumes of *0.1 M hydrochloric acid*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 250 mg of levodopa in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Centrifuge and use the supernatant liquid.

Reference solution (a). A solution containing 0.0025 per cent w/v, each of, *carbidopa IPRS, levodopa IPRS, levodopa impurity A IPRS, levodopa impurity B IPRS* and *methyldopa IPRS* in the solvent mixture.

Reference solution (b). A 0.0025 per cent w/v solution of *levodopa IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- sample temperature: 4°,
- mobile phase: a buffer solution prepared by dissolving 13.8 g of *potassium dihydrogen orthophosphate monohydrate* in 1000 ml of *water*, adjusted to pH 2.7 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Levodopa impurity A[1]	0.45	1.25
Levodopa	0.52	—
Methyldopa[2]	0.84	1.0
Carbidopa	1.0	—
Levodopa impurity B[3]	1.2	—
Carbidopa impurity A[4]	3.1	—
3,4- dihydroxyphenylacetone	3.9	1.0

[1](3-(3,4,6-Trihydroxyphenyl)alanine. Individual impurity based on the label claim of levodopa

[2]Individual impurity based on the label claim of levodopa and carbidopa.

[3](3-Methoxytyrosine) Process-related impurities, included for identification only; not to be included in Total impurities.

[4]Process-related impurities, included for identification only; not to be included in Total impurities.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to levodopa impurity A and levodopa is not less than 1.5, the resolution between the peaks due to carbidopa and levodopa impurity B is not less than 2 and the resolution between the peaks due to methyldopa and carbidopa is not less than 1.5.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to levodopa impurity A is not more than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.2 per cent), the area of any peak corresponding to methyldopa and 3,4-dihydroxyphenylacetone is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.5 per cent). The area of any other specified degradation impurity is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The sum of the areas of all the secondary peaks excluding 3,4- dihydroxyphenylacetone is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Test solution (a). Weigh and powder 10 tablets, disperse in the mobile phase with the aid of ultrasound and dilute to 1000.0 ml with the mobile phase.

Test solution (b). Dilute test solution (a) with the mobile phase to obtain a solution containing 0.025 per cent w/v of levodopa.

Reference solution. A solution of *carbidopa IPRS* having known concentration similar to the expected concentration of carbidopa in the test solution and 0.025 per cent w/v of *levodopa IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 6°,
- mobile phase: a mixture of 95 volumes of buffer solution prepared by dissolving 6.6 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.2 with *orthophosphoric acid* and 5 volumes of *ethanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

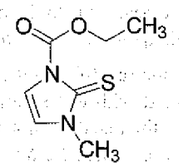
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.4 and the relative standard deviation of replicate injections is not more than 2.0 for levodopa and carbidopa peaks.

Inject the reference solution and test solution (b).

Calculate the content of $C_{10}H_{14}N_2O_4$ and $C_9H_{11}NO_4$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Carbimazole



$C_7H_{10}N_2O_2S$ Mol. Wt. 186.2

Carbimazole is ethyl 3-methyl-2-thioxo-4-imidazoline-1-carboxylate.

Carbimazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_{10}N_2O_2S$, calculated on the dried basis.

Category. Antithyroid.

Description. A white or creamy-white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbimazole IPRS* or with the reference spectrum of carbimazole.

B. In the test for Thiamazole and other related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (c).

C. To a solution prepared by dissolving about 10 mg in a mixture of 50 ml of *water* and 0.05 ml of *dilute hydrochloric acid*, add 1 ml of *potassium iodobismuthate solution*; a red precipitate is produced.

Tests

Thiamazole and other related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Solvent mixture. 20 volumes of *acetonitrile* and 80 volumes of *water*.

Test solution. Dissolve 5 mg of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution (a). Dilute 1.0 ml of 0.005 per cent w/v solution of *thiamazole* (carbimazole impurity A) in the solvent mixture, and 2.0 ml of test solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *thiamazole* in 10.0 ml of the solvent mixture. Dilute 1.0 ml of the solution to 100 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to carbimazole impurity A and carbimazole is not less than 5.0.

Inject reference solution (b) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with test solution, the area of secondary peak corresponding to carbimazole impurity A is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the areas of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent). Ignore any peak with area less than 0.05 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under test of Related substances with the following modifications.

Reference solution. Dissolve 5 mg of *carbimazole IPRS* to 10.0 ml with the solvent mixture.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_7H_{10}N_2O_2S$.

Storage. Store protected from light.

Carbimazole Tablets

Carbimazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carbimazole, $C_7H_{10}N_2O_2S$.

Usual strengths. 5 mg; 10 mg; 20 mg.

Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Carbimazole with two quantities, each of 5 ml of *chloroform*. Combine the chloroform extracts, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 30 minutes The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbimazole IPRS* or with the reference spectrum of carbimazole.

B. To a small quantity of the powdered tablets add 1 drop of *dilute potassium iodobismuthate solution*; a scarlet colour is produced.

Tests

Thiamazole and other related substances. Determine by liquid chromatography (2.4.14).

NOTE —Use freshly prepared solutions and protect from light.

Test solution. Disperse a quantity of the powdered tablets containing 20 mg of Carbimazole in 10.0 ml of *acetonitrile* with the aid of ultrasound for 5 minutes, filter. Dilute 1.0 ml of the solution to 20.0 ml with *water*.

Reference solution (a). A 0.00005 per cent w/v of *carbimazole IPRS* in 5 per cent v/v of *acetonitrile*.

Reference solution (b). A 0.0001 per cent w/v of *thiamazole* in 5 per cent v/v of *acetonitrile*.

Reference solution (c). A solution containing 0.002 per cent w/v of *carbimazole IPRS* and 0.0001 per cent w/v of *thiamazole* in 5 per cent v/v of *acetonitrile*.

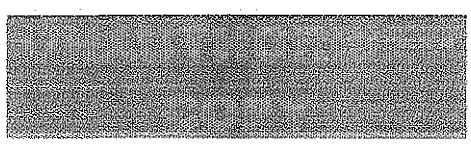
Chromatographic system

- a stainless steel column 15 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 5.0 per cent v/v solution of *acetonitrile*,
  B. 20.0 per cent v/v solution of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
4.6	100	0
30	0	100
30.1	100	0
40	100	0

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to thiamazole (carbimazole impurity A) and carbimazole is not less than 5.0.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with test solution, the area of any peak corresponding to thiamazole is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).



Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse one tablet in 5 ml of *water* with the aid of ultrasound for 5 minutes and dilute with mobile phase A to obtain a solution having concentration of 0.005 per cent w/v of Carbimazole.

Inject reference solution (a) and the test solution.

Calculate the content of $C_7H_{10}N_2O_2S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light and prepare immediately before use.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 20 mg of Carbimazole in 5 ml of *water,* with the aid of ultrasound for 5 minutes and dilute to 20.0 ml with *acetonetrile,* filter. Dilute 5.0 ml of the solution to 100.0 ml with mobile phase A.

Reference solution (a). A 0.005 per cent w/v solution of *carbimazole IPRS* in mobile phase A.

Reference solution (b). A solution containing 0.01 per cent w/v of *carbimazole IPRS* and 0.0005 per cent w/v of *thiamazole* in mobile phase A.

Use chromatographic conditions as described under test for Thiamazole and other related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to thiamazole (carbimazole impurity A) and carbimazole is not less than 5.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_7H_{10}N_2O_2S$ in the tablets.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Carbomers

Carbomers are high molecular mass polymers of acrylic acid cross-linked with polyalkenyl ethers of sugars or polyalcohols.

Carbomers contains not less than 56.0 per cent and not more than 68.0 per cent of carboxylic acid groups (-COOH), calculated on the dried basis.

Category. Excipient.

Description. A white, fluffy powder, hygroscopic.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carbomers IPRS* or with reference spectrum of carbomers.

B. Adjust a 1 per cent w/v dispersion to about pH 7.5 with *1 M sodium hydroxide.* A highly viscous gel is formed.

C. Add 2 ml of a 10 per cent w/v solution of *calcium chloride* with continuous stirring to 10 ml of the gel obtained in test B. A white precipitate is immediately produced.

D. Add 0.5 ml of *thymol blue solution* to 10 ml of a 1 per cent w/v dispersion. An orange colour is produced. Add 0.5 ml of *cresol red solution* to 10 ml of a 1 per cent w/v dispersion. A yellow colour is produced.

E. It complies with the test for viscosity (2.4.28).

Tests

Apparent viscosity. The nominal apparent viscosity is in the range 300 mPa s to 115000 mPa s. For a product with a nominal apparent viscosity of 20000 mPa s or greater, the apparent viscosity is 70.0 per cent to 130.0 per cent of the value stated on the label; for a product with a nominal apparent viscosity less than 20 000 mPa s, the apparent viscosity is 50.0 per cent to 150.0 per cent of the value stated on the label.

Dry the substance under examination in vacuum at 80° for 1 hour. Carefully add 2.5 g of the previously dried substance under examination to 500 ml of *water* in a 1000-ml beaker while stirring continuously at 1000 ± 50 rpm, with the stirrer shaft set at an angle of 60° to one side of the beaker. Add the previously dried substance over a period of 45 to 90 seconds, at a uniform rate, ensuring that loose aggregates of powder are broken up and continue stirring at 1000 ± 50 rpm for 15 minutes. Remove the stirrer, and place the beaker containing the dispersion in a water-bath at 25 ± 0.2° for 30 minutes. Insert the stirrer to a depth necessary to ensure that air is not drawn into the dispersion, and while stirring at 300 ± 25 rpm, titrate with a glass-calomel electrode system to pH 7.3 to 7.8 by adding a 18 per cent w/v solution of *sodium hydroxide* below the surface, determining the end-point potentiometrically (2.4.25). The total volume of the 18 per cent w/v solution of *sodium hydroxide* used is about 6.2 ml. Allow 2-3 minutes before the final pH determination. If the final pH exceeds 7.8, discard the preparation, and prepare another using a smaller amount of sodium hydroxide for titration. Return the neutralised preparation to the water-bath at 25° for 1 hour, then perform the viscosity determination without delay to avoid slight viscosity changes that occur 75 minutes after neutralisation. Determine the viscosity (2.4.28) with a rotating

viscometer with a spindle rotating at 20 rpm, using a spindle suitable for the expected apparent viscosity.

Free acrylic acid. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.125 g of the substance under examination in 25 ml of a 2.5 per cent w/v solution of *aluminium potassium sulphate*. Heat the suspension at 50° for 20 minutes with shaking. Then shake the suspension at room temperature for 60 minutes. Centrifuge and use the clear supernatant solution.

Reference solution. Dissolve 62.5 mg of *acrylic acid IPRS* in 100 ml of a 2.5 per cent w/v solution of *aluminium potassium sulphate*. Dilute 1.0 ml of the solution to 50.0 ml with 2.5 per cent w/v solution of *aluminium potassium sulphate*.

Chromatographic system
- a stainless steel column 12 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 0.136 g in 100 ml of *potassium dihydrogen phosphate*, adjusted to pH 2.5 using *dilute phosphoric acid*,
  B. equal volumes of a solution of 0.136 g of *potassium dihydrogen phosphate* in 100 ml of *water* and *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
8	100	0
9	0	100
20	0	100
21	100	0
30	100	0

Inject the reference solution and the test solution. The retention time for acrylic acid is about 6.0 minutes. The area of the peak in the chromatogram obtained with the test solution is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.25 per cent).

Benzene. Determine by gas chromatography (2.4.13).

Solvent mixture. Dissolve 0.1 g of *benzene* in 100 ml of *dimethyl sulphoxide*. Further dilute 1.0 ml of the solution to 100.0 ml with *water*. Further dilute 1.0 ml of the solution to 100.0 ml with *water*.

Test solution. Weigh 50.0 mg of the substance under examination, add 5.0 ml of *water* and 1.0 ml of *dimethyl sulphoxide*.

Reference solution. Weigh 50.0 mg of the substance under examination, add 4.0 ml of *water*, 1.0 ml of *dimethyl sulphoxide* and 1.0 ml of the solvent mixture.

Close the vials with a tight rubber membrane stopper coated with polytetrafluoroethylene and secure with an aluminium crimped cap. Shake to obtain a homogeneous dispersion.

Chromatographic system
- a capillary column 30 m x 0.53 mm, packed with cyanopropyl phenyl polysiloxane,
- temperature :
  column at 130°,
  inlet port and detector at 240°,
- flow rate: 30 ml per minute using nitrogen as the carrier gas.

Stratic head-space conditions which may be used:
- equilibration temperature 80°,
- equilibration time 60 minutes,
- transfer line temperature 90°.

Inject 1 µl of the gaseous phase of the reference solution and 1 µl of the gaseous phase of the test solution; repeat these injections twice more. Maximum relative standard deviation of the differences in area between the analyte peaks obtained from the 3 replicate pair injections of the reference solution and the test solution is 15 per cent. The test is not valid unless the relative standard deviation for replicate injections is not more than 15 per cent.

The mean area of the peak corresponding to benzene in the chromatograms obtained with the test solution is not more than half the mean area of the peak corresponding to benzene in the chromatograms obtained with the reference solution (2 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 4.0 per cent, determined on 1.0 g.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in vacuum at 80° for 60 minutes.

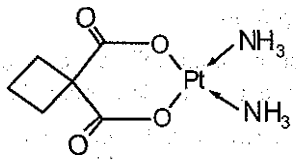
Assay. Weigh accurately about 0.12 g, add 50 ml of *water* slowly with stirring and heating at 60° for 15 minutes. Stop heating, add 150 ml of *water* and continue stirring for 30 minutes. Add 2 g of *potassium chloride* and titrate with *0.2 M sodium hydroxide* determining the end-point potentiometrically (2.4.25).

1 ml of *0.2 M sodium hydroxide* is equivalent to 0.009 g of carboxylic acid groups (-COOH).

Storage. Store protected from moisture.

Labelling. The label states the nominal apparent viscosity.

Carboplatin



$C_6H_{12}N_2O_4Pt$ Mol Wt .371.3

Carboplatin is (*SP*-4-2)-diammine[1,1-cyclo-butanedi (carboxylato-*kO*)(2-)]platinum.

Carboplatin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_6H_{12}N_2O_4Pt$, calculated on the dried basis.

Category. Anticancer.

Description. A colourless crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carboplatin IPRS* or with the reference spectrum of carboplatin.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *carbon dioxide free water* (Solution A) is clear and colourless (2.4.1).

Acidity and Impurity B. Not more than 0.5 per cent w/v, calculated as carboplatin impurity B (cyclobutane-1, 1-dicarboxylic acid). To 10 ml of solution A, add 0.1 ml of *phenolphthalein solution*, solution is colourless. Not more than 0.7 ml of *0.01 M sodium hydroxide* is required to change the colour of indicator to pink.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water.*

Test solution. Dissolve 20 mg of the substance under examination in 20.0 ml of the solvent mixture.

Reference solution. Dilute 0.5 ml of the test solution to 200.0 ml of with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with amino propylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 13 volumes of *water* and 87 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

The relative retention time with reference to carboplatin (retention time: about 7 minutes) for carboplatin impurity A (cis-diaminedichloroplatinum (II)) is about 0.3.

Inject the test solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to carboplatin impurity A is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.25 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with the area less than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Chlorides (2.3.12). Dissolve 0.4 g in *water*, heating slightly if necessary, and dilute to 15 ml with *water*, filter. The filtrate complies with the limit test of chlorides (100 ppm).Prepare the standard using 8.0 ml of chloride standard solution (5 ppm).

Ammonium. Not more than 100 ppm.

In a 25 ml jar fitted with a cap, dissolve or suspend 0.2 g of substance under examination in 1 ml of *water*. Add 0.3 g of *heavy magnesium oxide*. Close immediately after placing a piece of *silver manganese paper* 5 mm square, wetted with a few drops of *water*, under the polyethylene cap. Swirl, avoiding projections of liquid, and allow to stand at 40° for 30 minutes. If the silver manganese paper shows a grey colour, it is not more intense than that of a standard prepared at the same time and in the same manner using the prescribed volume of *ammonium standard solution*, 1 ml of *water* and 0.3 g of *heavy magnesium oxide*. Prepare the standard using 0.2 ml of *ammonium standard solution (100 ppm NH_4)*.

Silver. Not more than 10 ppm.

Determine by atomic emission spectrophotometry (2.4.3), measuring at 328.1 nm.

Test solution. Dissolve 0.5 g in a 1 per cent v/v solution of *nitric acid* and dilute to 50.0 ml with the same solution.

Reference solutions. Prepare the reference solutions using *silver standard solution (5 ppm Ag)*, diluting with a 1 per cent v/v solution of *nitric acid.*

Soluble barium. Not more than 10 ppm.

Determine by atomic emission spectrophotometry (2.4.3), measuring at 455.4 nm.

Test solution. Use the solution described under test for silver.

Reference solutions. Prepare the reference solutions using *barium standard solution (50 ppm Ba)*, diluting with 1.0 per cent v/v solution of *nitric acid.*

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Ignite 0.2 g of residue obtained in the test for loss on drying to a constant mass at 800°.

1 mg of the residue is equivalent to 0.001903 g of $C_6H_{12}N_2O_4Pt$.

Storage. Store protected from light.

Carboplatin Injection

Carboplatin Injection is a sterile solution of Carboplatin in Water for Injections.

Carboplatin Injection contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of carboplatin, $C_6H_{12}N_2O_4Pt$.

Usual strength. 10 mg per ml.

Identification

A. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel.*

NOTE—Carry out the test protected from light and use freshly prepared solution.

Mobile phase. A mixture of 20 volumes of *water* and 80 volumes of *acetone.*

Test solution. Dilute a volume of injection to obtain a solution containing 1.0 per cent w/v of Carboplatin in *water.*

Reference solution. A 1.0 per cent w/v solution of *carboplatin IPRS* in *water.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air for 2 hours. Spray the plate with a solution prepared immediately by dissolving 5.6 g of *tin(II)chloride* in 10.0 ml of *hydrochloric acid*, and dilute to 100 ml with *water*, add 1.0 g of *potassium iodide* and stir. Heat the plate at 100° for 10 minutes and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.0 to 7.0.

Cyclobutane-1,1-dicarboxylic acid. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light and use freshly prepared solution.

Test solution. Dilute a volume of injection to obtain a solution containing 0.1 per cent w/v of Carboplatin in *water.*

Reference solution (a). A 0.001 per cent w/v solution of *cyclobutane-1,1-dicarboxylic acid* in *water.*

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 2 volumes of buffer solution prepared by dissolving 8.5 g of *tetrabutylammonium hydrogen sulphate* in 80 volumes of *water*, add 3.4 ml of *orthophosphoric acid* and adjusted to pH 7.5 with *10 M sodium hydroxide*, 10 volumes of *acetonitrile* and 88 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 100 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peak corresponding to carboplatin and cyclobutane-1,1- dicarboxylic acid is not less than 2.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to cyclobutane-1,1- dicarboxylic acid is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Bacterial endotoxins (2.2.3). Not more than 5.4 Endotoxin Units per ml of 1.0 per cent w/v solution of Carboplatin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light and use freshly prepared solution.

Test solution. Dilute the injection with *water* if necessary to produce a solution containing 0.1 per cent w/v of Carboplatin.

Reference solution . A 0.1 per cent w/v solution of *carboplatin IPRS* in *water.*

Charomatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with aminopropylsilane bonded to porous silica (5 µm) (Such as µ Bondapak –NH_2),
- mobile phase: a mixture of 13 volumes of *water* and 87 volumes of *acetonitrile*,

– flow rate: 2 ml per minute,
– spectrophotometer set at 230 nm,
– injection volume: 20 µl.

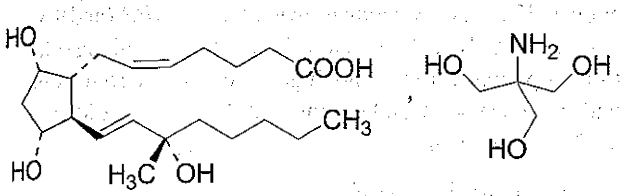
Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_6H_{12}N_2O_4Pt$ in the injection.

Storage. Store protected from light and free from contact with metals.

Carboprost Tromethamine



$C_{21}H_{36}O_5,C_4H_{11}NO_3$ Mol. Wt. 489.70

Carboprost Tromethamine is a salt of (5*Z*,13*E*)-(8*R*,9*S*,11*R*,12*R*,15*S*)-9,11,15-trihydroxy-15-methyl-prosta-5,13-dienoic acid with 2-amino-2-hydroxymethyl-1,3-propanediol.

Carboprost Tromethamine contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{21}H_{36}O_5,C_4H_{11}NO_3$, calculated on the dried basis.

CAUTION — Great care should be taken to prevent inhaling particles of Carboprost Tromethamine and exposing the skin to it.

Category. Uterine stimulant; abortifacient.

Description. A white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carboprost tromethamine IPRS* or with reference spectrum of carboprost tromethamine. Examine the substances as mulls.

Tests

Specific optical rotation. (2.4.22) + 18.0° to + 24.0°, determined in a 1.0 per cent w/v solution in *ethanol (95 per cent)*.

15*R*-Epimer and 5-*trans* isomer. Determine by liquid chromatography (2.4.14).

Follow the method described under Assay but using injection volume 25 µl. The usual order of elution is guaiphenesin, the 2-naphthacyl ester of 15*R*-epimer, the 2-naphthacyl ester of carboprost and the 2-naphthacyl ester of the *5-trans* isomer with retention times of about 7, 8, 11 and 13 minutes respectively. Measure the peak areas for the four components and calculate the contents of the 15*R*-epimer and *5-trans* isomer. The percentages of 15*R*-epimer (as tromethamine salt) and *5-trans* isomer are not more than 2.0 per cent and 4.0 per cent respectively.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined by drying in oven at 50° for 16 hours at a pressure not exceeding 0.7 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 5 mg of the substance under examination, transfer to a stoppered 50-ml centrifuge tube. Add 20.0 ml of *dichloromethane* and 2 ml of citrate buffer prepared by dissolving 10.5 g of *citric acid monohydrate* in about 75 ml of *water*, adjusted the pH of the solution to 4.0 by addition of *sodium hydroxide solution* slowly and diluting to 100 ml with *water*. Shake the stoppered tube for about 10 minutes and centrifuge. Transfer 4.0 ml of the lower dichloromethane layer to a suitable vial and evaporate the solvent with the aid of a stream of nitrogen. To the dried material add 100 µl of a freshly prepared 2 per cent w/v solution of *α-bromo-2'-acetonaphthone* in *acetonitrile* and swirl to wash down the sides of the vial. Add 50 µl of a freshly prepared 1 per cent v/v solution of *diisopropylethylamine* in *acetonitrile*, swirl again and place the vial at a temperature of 30° to 35° for not less than 15 minutes. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 2.0 ml of a 0.7 per cent w/v solution of guaiphenesin (internal standard) in the mobile phase, mix and filter the resulting solution through a fine porosity filter.

Reference solution. Prepare in the same manner but using *caroboprost tromethamine IPRS* in place of the substance under examination.

Chromatographic system
– stainless steel column 30 cm x 4 mm, packed with porous silica particles (3 to 10 µm),
– mobile phase: a mixture of 7 volumes of *1,3-butanediol*, 0.5 volume of *water* and 992 volumes of *dichloromethane*,
– flow rate: 1.5 ml per minute,
– spectrophotometer set 254 nm,
– injection volume: 10 µl.

The retention times for guaiphenesin and the 2-naphthacyl ester of carboprost are about 7 minutes and 11 minutes respectively.

Inject the reference solution. The test is not valid unless the resolution between these two peaks is greater than 4.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{36}O_5,C_4H_{11}NO_3$.

Storage. Store in a refrigerator (2° to 8°).

Carboprost Tromethamine Injection

Carboprost Tromethamine Injection is a sterile solution of Carboprost Tromethamine in Water for Injections. It may contain Benzyl alcohol, Sodium Chloride and Tromethamine.

Carboprost Tromethamine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carboprost, $C_{21}H_{36}O_5$.

Usual strengths. the equivalent of 250 µg and 500 µg of carboprost in 1 ml.

Description. A colourless solution.

Identification

Extract a volume of the injection containing 2.5 mg of Carboprost Tromethamine with 1.5 to 2 times its volume of *chloroform.* Discard the chloroform layer and acidify the aqueous layer with 3 to 5 drops of *hydrochloric acid.* Extract the acidified solution with an equivalent volume of *chloroform.* Filter the chloroform layer through a pledget of cotton and concentrate the filtrate to a volume of less than 1 ml. To the resulting solution add 150 mg to 180 mg of *potassium bromide IR* and mix well. Dry the potassium bromide mixture in vacuum overnight and prepare a disc from the dried mixture.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carboprost tromethamine IPRS* treated in the same manner or with reference spectrum of carboprost tromethamine.

Tests

pH (2.4.24). 7.0 to 8.0.

Bacterial endotoxins. Not more than 714.3 Endotoxin Units per mg of carboprost tromethamine.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer a volume of the injection containing 500 µg of carboprost to a stoppered 50-ml centrifuge tube. Add 20.0 ml of *dichloromethane* and 1.0 ml of citrate buffer prepared by dissolving 10.5 g of *citric acid monohydrate* in about 75 ml of *water,* adjusting the pH of the solution to 4.0 by addition of *sodium hydroxide solution* slowly and diluting to 100.0 ml with *water.* Shake the stoppered tube for about 10 minutes and centrifuge. Transfer 8.0 ml of the lower dichloromethane layer to a suitable vial and evaporate the solution with the aid of a stream of nitrogen (The residue may not evaporate to dryness because of the presence of benzyl alcohol). Add 100 µl of a freshly prepared 2 per cent w/v solution of *α-bromo-2'-acetonaphthone in acetonitrile* and swirl to wash down the sides of the vial. Add 50 µl of a freshly prepared 1 per cent v/v solution of *diisopropylethylamine* in *acetonitrile,* swirl again and place the vial at a temperature of 30° to 35° for not less than 15 minutes. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 1.0 ml of a 0.3 per cent w/v solution of *guaiphenesin* (internal standard) in the mobile phase, mix and filter the resulting solution through a fine porosity filter.

Reference solution. Prepare an aqueous solution containing about 0.332 mg of *carboprost tromethamine IPRS* and 9 mg of *benzyl alcohol* per ml. Transfer 2.0 ml of the resulting solution to a stoppered 50-ml centrifuge tube and proceed as given under the test solution beginning at the words "Add 20.0 ml *of dichloromethane.....".*

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with porous silica particles (3 to 10 µm),
- mobile phase: a mixture of 7 volumes of *1,3-butanediol,* 0.5 volume of *water* and 992 volumes of *dichloromethane,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set 254 nm,
- injection volume: 10 µl.

The retention times for guaiphenesin and the 2-naphthacyl ester of carboprost are about 7 minutes and 11 minutes respectively.

Inject the reference solution. The test is not valid unless the resolution between these two peaks is greater than 4.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

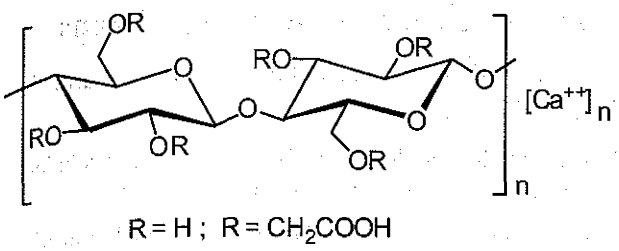
Calculate the quantity, in µg, of carboprost $C_{21}H_{36}O_5$ per ml of the injection from the ratios of the peak response of the 2-naphthacyl ester of carboprost and the internal standard obtained with the test solution, the ratios of the peak response of the 2-naphthacyl ester of carboprost and the internal standard obtained with the reference solution and the concentration, in µg per ml, of carboprost in *carboprost tromethamine IPRS* in the reference solution.

Storage. Store in a refrigerator (2° to 8°).

Labelling. The label states the strength in terms of the equivalent amount of carboprost in a suitable dose-volume.

Carboxymethylcellulose Calcium

Carmellose Calcium



R = H ; R = CH_2COOH

Carboxymethylcellulose Calcium is the calcium salt of a partially substituted poly(carboxymethyl) ether of cellulose.

Category. Pharmaceutical aid.

Description. A white to yellowish-white powder.

Identification

A. Shake 0.1 g thoroughly with 10 ml of *water*. Add 2 ml of *dilute sodium hydroxide solution* and allow to stand for 10 minutes (Solution A). Dilute 1 ml of solution A to 5 ml with *water*. To 0.05 ml of the solution, add 0.5 ml of a 0.05 per cent w/v solution of *chromotropic acid, sodium salt* in *sulphuric acid (75 per cent)* and heat on a water-bath for 10 minutes; a reddish-violet colour develops.

B. Shake 5 ml of solution A with 10 ml of *acetone*; a white, flocculent precipitate is produced.

C. Shake 5 ml of solution A with 1 ml of *ferric chloride solution*.; a brown, flocculent precipitate is formed.

D. Ignite 1 g and dissolve the residue in a mixture of 5 ml of *acetic acid* and 10 ml of *water*, boil for 5 minutes. Cool and neutralise with *dilute ammonia*. The solution gives reaction (a) of calcium (2.3.1).

Tests

Alkalinity. Shake 1.0 g thoroughly with 50 ml of *carbon dioxide-free water* and add 0.05 ml of *phenolphthalein solution*. No red colour develops.

Chlorides (2.3.12). Shake 1.0 g with 50 ml of *water*; add 5 ml of *dilute sodium hydroxide solution* and dilute to 100 ml with *water*. Heat 28 ml of the solution with 10 ml of *dilute nitric acid* on a water-bath until a flocculent precipitate is produced. Cool, centrifuge and separate the supernatant liquid. Wash the precipitate with 3 quantities, each of 10 ml of *water*, centrifuging each time. Combine the supernatant liquid and the washings and dilute to 100 ml with *water*. To 25 ml, add 6 ml of *dilute nitric acid* . This solution complies with the limit test for chlorides (0.36 per cent).

Sulphates (2.3.17). Shake 1.0 g with 50 ml of *water*, add 5 ml of *dilute sodium hydroxide solution* and dilute to 100 ml with *water*. Heat 20 ml of the solution with 1 ml of *hydrochloric acid* on a water-bath until a flocculent precipitate is produced. Cool, centrifuge and separate the supernatant liquid. Wash the precipitate with 3 quantities, each of 10 ml, of *water*, centrifuging each time. Combine the supernatant liquid and the washings and dilute to 100 ml with *water*. To 7.5 ml add 1 ml of *dilute hydrochloric acid* and dilute to 50 ml with *water*. The resulting solution complies with the limit test for sulphates (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). 10.0 per cent to 20.0 per cent.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Storage. Store protected from moisture.

Carboxymethylcellulose Sodium

Sodium Carboxymethylcellulose; Carmellose Sodium

Carboxymethylcellulose Sodium is the sodium salt of a partially-substituted poly(carboxymethyl) ether of cellulose.

Sodium Carboxymethylcellulose contains not less than 6.5 per cent and not more than 10.8 per cent of sodium, Na, calculated on the dried basis.

Category. Pharmaceutical aid.

Description. A white or almost white, granular powder; hygroscopic.

Identification

A. Sprinkle a quantity containing 1.0 g of the dried substance on to 90 ml of *carbon dioxide-free water* at 40° to 50°, stir vigorously until a colloidal solution is produced, cool and dilute to 100 ml with *carbon dioxide-free water* (solution A). To 10 ml of solution A add 1 ml of *copper sulphate solution*; a blue, cotton-like precipitate is produced.

B. Boil 5 ml of solution A for a few minutes; no precipitate is produced.

C. Solution A gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. Solution A is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 6.0 to 8.0, determined in solution A.

Apparent viscosity. 75 to 140 per cent of the declared value, determined by the following method. To 50 ml of water heated to 90° add, with stirring, a quantity containing 2 g of the dried substance under examination or, for a product of low viscosity, use the quantity required to give the concentration on the label. Allow to cool, dilute to 100 ml with *water* and continue stirring until solution is complete. Determine the viscosity by Method C (2.4.28), at 20° using a shear rate of 10 s^{-1}. If necessary, use rates slightly below and slightly above 10 s^{-1} and interpolate.

Arsenic (2.3.10). Place 5.0 g in a dry Kjeldahl flask, add 20 ml of *nitric acid*, and warm cautiously until the reaction commences. Allow the reaction to subside without further heating, then add a mixture of 20 ml of *nitric acid* and 5 ml of *sulphuric acid* and heat until brown fumes cease to be evolved. Add 0.5 ml of *perchloric acid (60 per cent)*, heat until white fumes appear, and if the liquid is still dark add further small quantities of *nitric acid* and heat until the liquid becomes pale yellow. Heat again until the white fumes appear and continue heating for a further 15 minutes. Add 0.5 ml of *perchloric acid (60 per cent)* and continue heating for a few minutes. Allow the solution to cool add 10 ml of *water*, and heat until white fumes appear. Repeat the heating with a further 5 ml of *water*, cool and add 40 ml of *water* and 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (1 ppm). Prepare the standard using 0.5 ml of *arsenic standard solution (10 ppm As)*.

Heavy metals (2.3.13). To the residue obtained in the test for Sulphated ash add 1 ml of *hydrochloric acid*, evaporate to dryness on a water-bath and dissolve the residue in 20 ml of *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D (20 ppm). Prepare the standard using *lead standard solution (1 ppm Pb)*.

Chlorides (2.3.12). 10 ml of solution A complies with the limit test for chlorides (0.25 per cent).

Sulphated ash (2.3.18). 20.0 to 33.3 per cent, calculated on the dried basis, determined on 1.0 g dispersed in a mixture of equal volumes of *sulphuric acid* and *water*.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.2 g and disperse in 80 ml of *anhydrous glacial acetic acid*. Heat on a water-bath for 2 hours, cool. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.002299 g of Na.

Storage. Store protected from light and moisture.

Labelling. The label states the apparent viscosity in mPas of a 2 per cent w/v solution or, where the viscosity is low, the concentration of the solution to be used and the apparent viscosity in mPas.

Carboxymethylcellulose Eye Drops

Carboxymethylcellulose Sodium Eye Drops; Carmellose Sodium Eye Drops

Carboxymethylcellulose Eye Drops are a sterile solution of Carboxymethylcellulose Sodium in Purified Water.

Carboxymethylcellulose Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carboxymethylcellulose Sodium.

Usual strength. 0.5 per cent w/v.

Identification

A. To 2 ml, add 1 ml of *water* to a test-tube, 5 drops of *1-napthol TS* and 2 ml of *sulphuric acid* to the test tube; red or purple colour is observed.

B. To 10 ml, add 5 ml of *barium chloride solution TS*; a white precipitate is formed.

C. To 4 ml, add 2 ml of 15 per cent w/v *potassium carbonate* solution and heat to boiling, no precipitate is formed. Add 4 ml of *potassium pyroantimonate TS*, heat to boil. Allow to cool in ice water; a dense precipitate is formed. Expose precipitate to non-luminous flame, the precipitate imparts an intense yellow colour to the flame.

Tests

pH(2.4.24). 5.0 to 8.0.

Other tests. Comply with the tests stated under Eye Drops.

Assay.

Solution A. A 20.0 per cent w/v solution of *polyhexanide* (vantocil 1B solution). Dilute 1.0 ml of the solution to 200 ml with *water*.

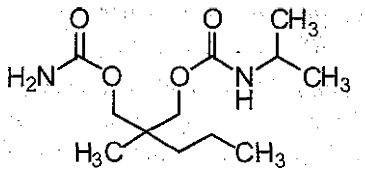
Test solution. To 5 ml, add sufficient *water* to produce 25 ml. To 5.0 ml of the solution, add 5 ml of solution A drop by drop while swirling the flask and dilute to 250.0 ml with *water*.

Reference solution. A 0.11 per cent w/v solution of *carboxymethylcellulose sodium IPRS* in *water*. To 5.0 ml of the solution, add 5 ml of solution A drop by drop while swirling the flask and dilute to 250.0 ml with *water*.

Measure the absorbance at the maximum at about 235 nm (2.4.7) using 5 ml of solution A diluted to 250 ml with *water* as the blank and calculate the content of carboxymethylcellulose sodium in the eye drops.

Storage. Store protected from light.

Carisoprodol



$C_{12}H_{24}N_2O_4$ Mol. Wt. 260.3

Carisoprodol is (*RS*)-2-{[(Aminocarbonyl)oxy]methyl}-2-methylpentylisopropylcarbamate.

Carisoprodol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{24}N_2O_4$, calculated on the dried basis.

Category. Muscle relaxant.

Description. A white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carisoprodol IPRS* or with the reference spectrum of carisoprodol.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Dissolve 0.5 g of Carisoprodol in the solvent mixture and dilute to 50.0 ml with the solvent mixture, with the aid of ultrasound.

Reference solution (a). A 0.01 per cent w/v solution of *carisoprodol IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0125 per cent w/v, each of, *carisoprodol impurity A IPRS*, *meprobamate IPRS* and *carisorprodol IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (4µm),
- mobile phase: A. a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 25 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
35	100	0
36	80	20
51	80	20
52	100	0
60	100	0

Name	Relative retention time	Correction factor
Carisoprodol impurity A[1]	0.19	16.7
Meprobamate	0.24	12.5
Carisoprodol monocarbamate[2]	0.86	0.71
Carisoprodol	1.0	—

[1]2-hydroxymethyl-2-methylpentyl carbamate,

[2]N-Isopropyl-2-hydroxymethyl-2-methylpentyl carbamate.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to carisoprodol impurity A and meprobamate is not less than 1.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to carisoprodol impurity A and carisoprodol monocarbamate is not more than 0.1 times the area of the principal peak obtained in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any peak due to meprobamate is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 60° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances using following modifications.

Test solution. Dissolve 25 mg of Carisoprodol in the solvent mixture and dilute to 10.0 ml with the solvent mixture with the aid of ultrasound.

Reference solution. A 0.25 per cent w/v solution of *carisoprodol IPRS* in the solvent mixture.

Inject the reference solution and the test solution.

Calculate the content of $C_{12}H_{24}N_2O_4$.

Storage. Store protected from moisture.

Carisoprodol Tablets

Carisoprodol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carisoprodol, $C_{12}H_{24}N_2O_4$.

Usual strength. 350 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

NOTE—Use only freshly prepared solutions containing -amylase; and equilibrate the dissolution medium at 37° for not more than one hour before beginning the dissolution test.

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.05 M phosphate buffer pH 6.9*, containing 5 units of a-amylase per ml,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14), as described under Assay using 150 µl injection volume:

Test solution. Use the filtrate, dilute if necessary, with *0.05 M phosphate buffer pH 6.9.*

Reference solution. A 0.04 per cent w/v solution of *carisoprodol IPRS* prepared by dissolving in minimum quantity of *acetonitrile* and suitably diluting with *0.05 M phosphate buffer pH 6.9.*

Inject the reference solution and the test solution.

Calculate the content of $C_{12}H_{24}N_2O_4$.

Q. Not less than 80 per cent of the stated amount of $C_{12}H_{24}N_2O_4$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *methanol* and 40 volumes of *0.005 M sulphuric acid.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.35 g of Carisoprodol in the solvent mixture with the aid of ultrasound for 30 minutes and mechanically shaking for 60 minutes, dilute to 100.0 ml with the solvent mixture, and filter.

Reference solution (a). A 0.35 per cent w/v solution of *carisoprodol IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.24 per cent w/v of *2-methyl-2-propyl-1,3-propanediol IPRS* and 0.34 per cent w/v of *carisoprodol IPRS* in the mobile phase.

Charomatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile,*
- flow rate: 2 ml per minute,
- refractive index detector maintained at 30°,
- injection volume: 35 µl.

The relative retention time with respect to carisoprodol for 2-methyl-2-propyl-1,3-propanediol is about 0.5.

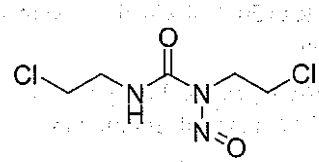
Inject reference solution (a) and (b). The test is not valid unless the resolution between the 2-methyl-2-propyl-1,3-propanediol and carisoprodol peaks is not less than 2.0 in the chromatogram obtained with reference solution (b) and in the chromatogram obatined with reference solution (a) the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{12}H_{24}N_2O_4$ in the tablets.

Storage. Store protected from moisture.

Carmustine



$C_5H_9Cl_2N_3O_2$ Mol. Wt. 214.1

Carmustine is urea, *N,N'*-bis(2-chloroethyl)-*N*-nitroso-;1,3-bis(2-chloroethyl)-1-nitrosourea.

Carmustine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_5H_9Cl_2N_3O_2$, calculated on the anhydrous and solvent- free basis.

Category. Anticancer.

Description. Light yellow powder.

CAUTION — Use appropriate surgical gloves, arm covers and a dust mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Melt a sufficient quantity of sample between 33° to 40° in a water-bath or oven, prepare a thin film between two previously warmed bromide plates and record the spectrum immediately. Compare the spectrum with that obtained with *carmustine IPRS* treated in the same manner or with the reference spectrum of carmustine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution.

Tests

Related substances (Carmustine Related Compound A). Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solution in low-actinic glassware and keep them refrigerated until use.

Test solution. Disperse 15.0 mg of substance under examination in *acetonitrile* and dilute to 10.0 ml with *acetonitrile.*

Reference solution (a). A 0.15 per cent w/v solution of *carmustine IPRS* in *acetonitrile.*

Reference solution (b). A 0.00075 per cent w/v solution of *carmustine related compound A IPRS [1-3-Bis(2-chloroethyl) urea]* in *acetonitrile.*

Reference solution (c). Dilute reference solution (b) to obtain a solution of 0.000075 per cent w/v solution of *carmustine related compound A IPRS* in *acetonitrile.*

Reference solution (d). A solution containing 0.00075 per cent w/v, each of, *carmustine IPRS* and *carmustine related compound A IPRS* in *acetonitrile.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 5°,
- mobile phase: a mixture of 70 volumes of *water* and 30 volumes of *acetonitrile,*
- flow rate: 1.5 ml per minute,
- spectrophometer set at 200 nm,
- injection volume: 10 µl.

The relative retention time with reference to carmustine for carmustine related compound A is about 0.3.

Inject reference solution (d). The test is not valid unless the resolution between the peaks corresponding to carmustine related compound A and carmustine is not less than 10.0.

Inject reference solution (a) and (c). The test is not valid unless the tailing factor is not more than 1.9 in the chromatogram obtained with reference solution (a) and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (c).

Inject reference solution (b) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to carmustine related compound A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Ether-insoluble substances. Not more than 0.1 per cent w/w, determined by the following method.

NOTE — Perform in a well-ventilated fume hood.

Weigh 1.0 g of sample and add to 10 ml of *anhydrous ether* in a suitable flask. Stir the solution for 5 minutes and immediately filter the solution through a tared sintered-glass filter, wash the flask with an additional 10 ml of *ether* and filter the solution through the same tared sintered-glass filter, dry to constant weight at 105° for 1 hour, cool in a desiccator and weigh.

2-Chloroethylamine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

NOTE — Prepare the solution in low-actinic glassware and keep them refrigerated until use.

Mobile phase (a). Ethyl acetate.

Mobile phase (b). A mixture of 70 volumes of *ethyl acetate* and 30 volumes of *methanol.*

Test solution. A 40.0 per cent w/v solution of carmustine in *methanol.*

Reference solution (a). A 0.12 per cent w/v solution of *2-chloroethylamine hydrochloride* in *methanol.*

Reference solution (b). A 0.04 per cent w/v solution of *carmustine IPRS* in *methanol.*

Apply 1 µl of each solution. Develop the chromatograms using separately the two mobile phases. Develop the chromatograms for 30 minutes using mobile phase (a), dry the plate in a current of air. Develop the plate again using mobile phase (b) for 10 minutes, dry the plate in a current of air and spray with *diethylamine solution.* Place the plate in an oven maintained at 100° for 20 minutes. Cool and spray again with *0.1M silver nitrate solution*, place the plate to be exposed under ultraviolet light at 365 nm for 15 minutes and examine under ultraviolet light at 365 nm. Any corresponding spot in the chromatograms obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent) and not more than two such spots are more intense than the spot in the chromatograms obtained with reference solution (b) (0.1 per cent).

2-Chloroethanol. Not more than 0.1 per cent w/w, determine by gas chromatography (2.4.13).

NOTE — Prepare the solution in low-actinic glassware and keep them refrigerated until use.

Test solution. A 1.0 per cent w/v solution of carmustine in *acetonitrile.*

Reference solution (a). A 0.002 per cent w/v solution of *2-chloroethanol* in *acetonitrile.*

Reference solution (b). Dilute reference solution (a) to obtain a 0.001 per cent w/v solution *2-chloroethanol* in the *acetonitrile.*

Chromatographic system

- a capillary column 30 m x 0.53 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of polyethylene glycol (Such as Carbowax 20 M),
- temperature:

column	time (min.)	temperature (°)
	0-6	40
	6-8	40 → 80
	8-22	80
	22-26	80 → 200
	26-29	200

- injector port: 90° and detector port at 260°,
- a flame ionisation detector,
- flow rate: 7 ml per minute, using nitrogen or helium as the carrier gas.

Inject 5 µl of reference solution (b).The test is not valid unless and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of 2-chloroethanol.

Acetaldehyde. Not more than 0.1 per cent w/w, determine by gas chromatography (2.4.13).

NOTE — Prepare the solution in low-actinic glassware and keep them refrigerated until use.

Test solution. A 1.0 per cent w/v solution of Carmustine in *acetonitrile.*

Reference solution. A 0.001 per cent w/v solution *acetaldehyde* in the *acetonitrile.*

Chromatographic system

- a capillary column 30 m x 0.53 mm packed with dimethylpolysiloxane (film thickness 5.0 µm),
- temperature:

column	time (min.)	temperature (°)
	0-6	40
	6-12	40 → 210
	12-15	210

- injector port: 70° and detector port at 260°,
- a flame ionisation detector,
- split ratio: 15:1,
- flow rate: 3 ml per minute, using nitrogen or helium as the carrier gas.

Inject 5 µl of the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of acetaldehyde.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solution in low-actinic glassware and keep them refrigerated until use.

Test solution. Disperse 15.0 mg of the substance under examination in *acetonitrile* and dilute to 10.0 ml with *acetonitrile.*

Reference solution. A 0.15 per cent w/v solution of *carmustine IPRS* in *acetonitrile.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 5°,
- mobile phase: a mixture of 70 volumes of *water* and 30 volumes of *acetonitrile,*
- flow rate: 1.5 ml per minute,
- spectrophometer set at 200 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.9 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_5H_9Cl_2N_3O_2$.

Storage. Store protected from light and moisture in a refrigerator between 2° to 8°.

Carmustine Injection

Carmustine Injection is a sterile lyophilized preparation of Carmustine.

The injection is prepared immediately before use by dissolving the contents of the sealed container which contains Carmustine with or without auxillary substances in a suitable diluent and then diluting with the requisite volume of a suitable diluent in accordance with the manufacturer's instructions.

The constituted solution complies with the requirements for the Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Carmustine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carmustine, $C_5H_9Cl_2N_3O_2$.

Usual strength. 100 mg.

CAUTION — Use appropriate surgical gloves, arm covers and a dust mask. Perform all work under a fume hood approved for testing of cytotoxic agents when possible.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Melt a sufficient quantity of sample between 33° to 40° in a water-bath or oven, prepare a thin film between two previously warmed bromide plates and record the spectrum immediately. Compare the spectrum with that obtained with *carmustine IPRS* treated in the same manner or with the reference spectrum of carmustine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

pH (2.4.24).4.0 to 6.8 of the constituted solution prepared as directed in the labelling.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions in low-actinic glassware and keep them refrigerated until use.

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of *water.*

Test solution (a). Dissolve a suitable quantity of the substance under examination in *acetonitrile* to obtain a solution containing 0.2 per cent w/v of Carmustine. (*NOTE — Allow test vials to warm to room temperature in desiccator for 1 hour).*

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of *carmustine IPRS* in *acetonitrile.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c). A 0.01 per cent w/v solution of *carmustine related compound A IPRS [1-3-Bis(2-chloroethyl) urea]* in *acetonitrile.*

Reference solution (d). Dilute reference solution (a) and (c) to obtain a solution containing 0.02 per cent w/v of *carmustine IPRS* and 0.0002 per cent w/v of *carmustine related compound A IPRS* with the solvent mixture, respectively.

Reference solution (e). Dilute reference solution (c) to obtain a solution containing 0.0002 per cent w/v of *carmustine related compound A IPRS* with the solvent mixture.

Chromatographic system

- a stainless steel column 7.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- sample temperature: 5°,
- mobile phase: A. *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophometer set at 200 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
2.5	90	10
7	40	60
8.5	90	10
10.5	90	10

The relative retention time with reference to carmustine for carmustine related compound A is about 0.5.

Inject reference solution (d). The test is not valid unless the resolution between the peaks corresponding to carmustine related compound A and carmustine is not less than 2.0, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent of the carmustine and carmustine related compound A peaks.

Inject reference solution (e) and test solution (b). In the chromatogram obtained with the test solution (b), the area of the peak due to carmustine related compound A is not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (1.0 per cent).

Water (2.3.43). Not more than 1.0 per cent.

Bacterial endotoxins (2.2.3). Not more than 0.95 Endotoxin Unit per mg of carmustine.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

NOTE — Prepare solution in low-actinic glassware and keep them refrigerated until use.

Inject reference solution (b) and test solution (b).

Calculate the content of $C_5H_9Cl_2N_3O_2$ in the injection..

Storage. Store protected from light and moisture in a refrigerator between 2° to 8°.

Labelling. The label on the sealed container states (1) the equivalent amount of carmustine contained in it; (2) that it should be used immediately after preparation.

Carnauba Wax

Carnauba Wax is obtained from the leaves of *Copernicia cerifera* Mart. (Fam. Palmae) after purification to remove foreign matter.

Category. Pharmaceutical aid.

Description. A pale yellow to light brown coarse powder, flakes or lumps of hard brittle wax; odour, characteristic and free from rancidity.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 98 volumes of *chloroform* and 2 volumes of *ethyl acetate*.

Test solution. Dissolve 0.1 g of the substance under examination, with warming, in 5 ml of *chloroform* and use the warm solution.

Reference solution. Dissolve 5 mg of (+)-*menthol*, 5 µl of *menthyl acetate* and 5 mg of *thymol* in 10 ml of *toluene*.

Apply separately to the plate, as bands 20 mm x 3 mm, 10 µl of reference solution and 30 µl of test solution.

After development, dry the plate in air and spray with a freshly prepared 20 per cent w/v solution of *phosphomolybdic acid* in *ethanol (95 per cent)* and heat at 105° for 15 minutes. The chromatogram obtained with the reference solution shows in the lower part a dark blue band due to menthol, a reddish band above it due to thymol and a dark blue band in the upper part due to menthyl acetate. The chromatogram obtained with the test solution shows a large blue band due to triacontanol (melissyl alcohol) at an R_f value between those of the bands due to menthol and thymol in the chromatogram obtained with the reference solution and blue bands at R_f values between those of the bands due to menthyl acetate and thymol in the chromatogram obtained with the reference solution. In addition, the chromatogram obtained with the test solution shows further bands at higher R_f values than menthyl acetate, that with the highest R_f value being very pronounced, and a number of faint bands below that due to triacontanol; a band on the line of application is blue.

Tests

Melting range (2.4.21). 78° to 88°, determined by Method II.

Acid value. Not more than 12.0, determined by the following method. Weigh accurately about 2.0 g *(w)* in a flask fitted with a reflux condenser, add 40 ml of *xylene* and heat until the substance has dissolved. Add 20 ml of *ethanol (95 per cent)* and titrate the hot solution with *0.5 M ethanolic potassium hydroxide*, using *phenolphthalein solution* as indicator, until a pink colour persists for at least 10 seconds (n_1 ml). Repeat the operation without the substance under examination (n_2 ml). Calculate the acid value from the expression $28.05(n_1-n_2)/w$.

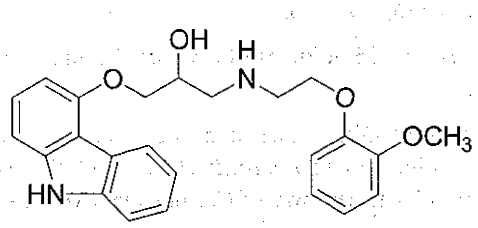
Saponification value. Between 78 and 95, determined by the following method. To the titrated solution from the determination of the Acid value, add 20.0 ml of *0.5 M ethanolic potassium hydroxide* and boil under a reflux condenser for 3 hours. Titrate the hot solution immediately with *0.5 M hydrochloric acid*, using 1 ml of *phenolphthalein solution* as indicator, until the red colour is discharged. Reheat the solution to boiling and continue the titration, if necessary, until the red colour no longer reappears on heating (n_3 ml). Repeat the operation without the substance under examination (n_4 ml). Calculate the saponification value from the expression $a+[28.05(n_4-n_3)/w]$ where a is the acid value.

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

Sulphated ash (2.3.18). Not more than 0.25 per cent, determined on 2.0 g.

Storage. Store protected from light and moisture.

Carvedilol



$C_{24}H_{26}N_2O_4$ Mol. Wt. 406.5

Carvedilol is (*RS*)-1-(9*H*-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol.

Carvedilol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{24}H_{26}N_2O_4$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *carvedilol IPRS* or with the reference spectrum of carvedilol.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25.0 mg of the substance under examination in sufficient mobile phase to produce 25.0 ml.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 0.005 g of *1-[[9-[2-hydroxy-3-[[2-(2-methoxyphenoxy)ethyl] amino]propyl]-9H-carbazol-4-yl]oxy]-3-[[2-(2-methoxyphenoxy)ethyl] amino]propan-2-ol (carvedilol impurity A) IPRS* in 5.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (Such as YMC- Pack pro C8),
- column temperature: 55°,
- mobile phase: dissolve 1.77 g of *potassium dihydrogen phosphate* in *water* and dilute to 650 ml with the same solvent, adjusted to pH 2.0 with dilute *orthophosphoric acid* and add 350 ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to carvedilol and carvedilol impurity A is not less than 1.7.

Inject the reference solution and the test solutions. Run the chromatograms 8 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to carvedilol impurity A is not more than twice the area of the peak in the chromatogram obtained with reference solution (c) (0.02 per cent), the area of the peak due to any other impurity is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than five times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.01 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm Pb).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.35 g and dissolve in 60 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04065 g of $C_{24}H_{26}N_2O_4$.

Storage. Store protected from light, at a temperature not exceeding 30°.

Carvedilol Tablets

Carvedilol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of carvedilol, $C_{24}H_{26}N_2O_4$.

Usual strengths. 3.125 mg; 6.25 mg; 12.5 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium. 900 ml of gastric buffer pH 1.3 prepared by dissolving 2 g of *sodium chloride* in 7 ml of *hydrochloric acid* and 500 ml of *water* and diluting to 1000 ml with *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14) using the chromatographic conditions as described under Assay.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Prepare a solution using *carvedilol IPRS* in the dissolution medium to obtain the same concentration as expected in the test solution.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{26}N_2O_4$.

Q. Not less than 70 per cent of the stated amount of $C_{24}H_{26}N_2O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 25 mg of Carvedilol with 15 ml of the mobile phase, dilute to 25 ml with the mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of *carvedilol IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (Such as YMC-Pack pro C8),
- column temperature: 55°,
- mobile phase: dissolve 1.77 g of *potassium dihydrogen phosphate* in *water*, dilute to 650 ml with the same solvent, adjusted to pH 2.0 with dilute *orthophosphoric acid* and add 350 ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the column efficiency in not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. Run the chromatograms 8 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions as described under Assay.

Test solution. Disperse one tablet in 5 ml of *water* and dilute to 25.0 ml with the mobile phase and filter.

Reference solution. Prepare a solution using *carvedilol IPRS* in the mobile phase to obtain the same concentration as expected in the test solution.

Calculate the content of $C_{24}H_{26}N_2O_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Carvedilol, disperse in 10 ml of *water*. Shake by hand add 35 ml of the mobile phase, mix with the aid of ultrasound for 30 minutes and shake on a mechanical shaker for about 30 minutes and dilute to 50 ml with the mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *carvedilol IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as YMC-Pack pro C8),
- mobile phase: a mixture of 50 volumes of 2 per cent w/v solution of *sodium heptane sulphonate* in *water*, 25 volumes of *acetonitrile* and 25 volumes of *methanol*, adjusted to pH 3.1 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 10 µl.

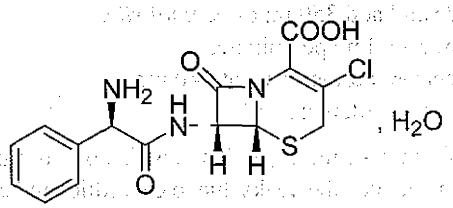
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{26}N_2O_4$ in the tablets.

Storage. Store protected from light, at a temperature not exceeding 30°.

Cefaclor



$C_{15}H_{14}ClN_3O_4S,H_2O$ Mol. Wt 385.8

Cefaclor is (6*R*,7*R*)-7-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene -2-carboxylic acid monohydrate.

Cefaclor contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{15}H_{14}ClN_3O_4S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or slightly yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefaclor IPRS* or with the reference spectrum of cefaclor.

Tests

pH (2.4.24). 3.0 to 4.5, determined in a suspension, prepared by dispersing 0.25 g in 10 ml of *carbon dioxide-free water*.

Specific optical rotation (2.4.22). +101° to +111°, determined in 1.0 per cent w/v solution in a 1.0 per cent w/v solution of *hydrochloric acid*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.27 per cent w/v solution of *sodium dihydrogen phosphate*, adjusted to pH 2.5 with *phosphoric acid*.

Test solution. Dissolve 50 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a) A solution containing 0.0025 per cent w/v *cefaclor IPRS* and 0.005 per cent w/v *delta-3-cefaclor IPRS* in the solvent mixture.

Reference solution (b) Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen phosphate* adjusted to pH 4.0 with *phosphoric acid*,
  B. mix 45 ml of *acetonitrile* with 55 ml of mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Increase the concentration of mobile phase B continuously and linearly by 0.67 per cent v/v per minute for 30 minutes (25 per cent v/v). Then increase the concentration of mobile phase B continuously and linearly by 5 per cent v/v per minute for 15 minutes (100 per cent v/v). Finally elute with mobile phase B for 10 minutes.

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes between each analysis. Inject the solutions. At the end of the programme change the composition of the mobile phase to a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A to re-equilibrate the column.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0 and the tailing factor of the cefaclor peak is not more than 1.2. If necessary, adjust the *acetonitrile* content of the mobile phase.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak, other than the principal peak and any peaks due to the mobile phase, is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all such peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). 3.0 to 6.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 15 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A 0.03 per cent w/v solution of *cefaclor IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.03 per cent w/v, each of, *cefaclor IPRS* and *delta-3-cefaclor IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture prepared by adding 22 volumes of *methanol* to a mixture of 78 volumes of *water*, 1 volume of *triethylamine* and 0.1 g of *sodium pentanesulphonate*, adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5 and the tailing factor of the cefaclor peak is not more than 1.5. Adjust the concentration of methanol in the mobile phase, if necessary.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{14}ClN_3O_4S$.

Storage. Store protected from moisture.

Cefaclor Capsules

Cefaclor Capsules contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cefaclor, $C_{15}H_{14}ClN_3O_4S$.

Usual strengths. 250 mg; 500 mg.

Identification

A. Shake a quantity of the contents of the capsules containing 0.3 g of anhydrous cefaclor with 100 ml of *water*, filter and dilute 1 ml of the filtrate to 100 ml with *water*.

When examined in the range 190 nm to 310 nm (24.7), the resulting solution shows an absorption maximum only at about 264 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*,

Speed and time. 50 rpm and 45 minute.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 264 nm (2.4.7). Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the medium from the absorbance obtained from a solution of known concentration of *cefaclor IPRS* in the same medium.

Q. Not less than 70 per cent of the stated amount of $C_{15}H_{14}ClN_3O_4S$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.27 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted pH to 2.5, if necessary, with *orthophosphoric acid*.

Test solution. Shake a quantity of the contents of the capsules containing 0.5 g of anhydrous cefaclor with 200 ml of the solvent mixture, dilute to 250 ml with the solvent mixture and filter.

Reference solution (a). A 0.002 per cent w/v solution of *cefaclor IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0025 per cent w/v of *cefaclor IPRS* and 0.005 per cent w/v of *delta-3-cefaclor IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS-2),
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 4.0 with *orthophosphoric acid*,

  B. a mixture of 45 volumes of *acetonitrile* and 55 volumes of mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
30	75	25
45	0	100
55	0	100
70	95	5

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the contents of capsules containing about 75 mg of anhydrous cefaclor with 200 ml of the mobile phase, dilute to 250.0 ml with the mobile phase and filter.

Reference solution (a). A 0.03 per cent w/v solution of *cefaclor IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.03 per cent w/v, each of, *cefaclor IPRS* and *delta-3-cefaclor IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Beckman Ultrasphere ODS and Supelcosil LC-18-DB),

- mobile phase: a solution prepared by dissolving 0.1 g of *sodium pentanesulphonate* in a mixture of 78 volumes of *water* and 1 volume of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid* adding 220 volumes of *methanol* and mixing,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the capsules.

Storage. Store protected from moisture.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of anhydrous cefaclor.

Cefaclor Oral Suspension

Cefaclor Oral Suspension is a mixture consisting of Cefaclor with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Cefaclor Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefaclor, $C_{15}H_{14}ClN_3O_4S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cefaclor, $C_{15}H_{14}ClN_3O_4S$.

Usual strengths. 125 mg per 5 ml; 250 mg per 5 ml.

Storage. Store protected from moisture at a temperature not exceeding 30°.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Identification

A. Shake a quantity of the oral suspension containing 0.3 g of anhydrous cefaclor with 500 ml of *water* and filter.

When examined in the range 190 nm to 310 nm (2.4.7), the filtrate shows an absorption maximum only at about 264 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.27 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5 with *orthophosphoric acid*.

Test solution. Shake a quantity of the oral suspension containing about 0.25 g of anhydrous cefaclor with 200 ml of the solvent mixture, dilute to 250 ml with the solvent mixture and filter.

Reference solution (a). A 0.001 per cent w/v solution of *cefaclor IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0025 per cent w/v of *cefaclor IPRS* and 0.005 per cent w/v of *delta-3-cefaclor IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS-2),
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 4.0 with *orthophosphoric acid*,
  B. a mixture of 45 volumes of *acetonitrile* and 55 volumes of mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
30	75	25
45	0	100
55	0	100
70	95	5

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes.

Inject reference solution (b). The test is not valid unless resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of the areas of any such peaks is not greater than three times the area of the principal peak in the chromatogram obtained with reference solution (a)

(3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the oral suspension containing about 75 mg of anhydrous cefaclor with 200 ml of the mobile phase, dilute to 250.0 ml with the mobile phase and filter.

Reference solution (a). A 0.03 per cent w/v solution of *cefaclor IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.03 per cent w/v, each of, *cefaclor IPRS* and *delta-3-cefaclor IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Beckman Ultrasphere ODS and Supelcosil LC-18-DB),
- mobile phase: a solution prepared by dissolving 0.1 g of *sodium pentanesulphonate* in a mixture of 78 volumes of *water* and 1 volume of *triethylamine*, adjusted to pH 2.5 using *orthophosphoric acid*, adding 22 volumes of *methanol* and mixing,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5 and the tailing factor of the peak due to cefaclor is not more than 1.5.

Inject reference solution (a) and the test solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{15}H_{14}ClN_3O_4S$, weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Storage. Store at the temperature and use within the period stated on the label.

Labelling. The label states the quantity in terms of the equivalent amount of anhydrous cefaclor.

Cefaclor Prolonged-release Tablets

Cefaclor Sustained-release Tablets; Cefaclor Extended-release Tablets

Cefaclor Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Cefaclor Prolonged-release Tablets contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of cefaclor, $C_{15}H_{14}ClN_3O_4S$.

Usual strengths. 125 mg; 250 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.3 g of anhydrous cefaclor with 100 ml of *water*, filter and dilute 1 ml of the filtrate to 100 ml with *water*.

When examined in the range 190 nm to 310 nm (2.4.7), the resulting solution shows an absorption maximum at about 264 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2). Complies with the test stated under tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.27 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted pH to 2.5, if necessary, with *orthophosphoric acid*.

Test solution. Shake a quantity of the powdered tablets containing 0.75 g of anhydrous cefaclor with 200 ml of the solvent mixture, dilute to 250 ml with the solvent mixture and filter.

Reference solution (a). A 0.003 per cent w/v solution of *cefaclor IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0025 per cent w/v of *cefaclor IPRS* and 0.005 per cent w/v of *delta-3-cefaclor IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS-2),
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 4.0 with *orthophosphoric acid*,
  B. a mixture of 45 volumes of *acetonitrile* and 55 volumes of mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
30	75	25
45	0	100
55	0	100
70	95	5

Equilibrate the column with a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A for at least 15 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.0. If necessary, adjust the proportion of acetonitrile in the mobile phase.

Inject reference solution (a) and the test solution. In the chromatogram obtained with test solution the area of any secondary peak is not greater than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent) and the sum of the areas of any such peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 75 mg of anhydrous cefaclor, disperse in the mobile phase, shake, dilute to 250.0 ml with the mobile phase and filter.

Reference solution (a). A 0.03 per cent w/v solution of *cefaclor IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.03 per cent w/v, each of, *cefaclor IPRS* and *delta-3-cefaclor IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Beckman Ultrasphere ODS and Supelcosil LC-18-DB),
- mobile phase: a solution prepared by dissolving 0.1 g of *sodium pentanesulphonate* in a mixture of 78 volumes of *water* and 1 volume of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid*, adding 22 volumes of *methanol* and mixing,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefaclor and delta-3-cefaclor is not less than 2.5.

Inject reference solution (a) and the test solution.

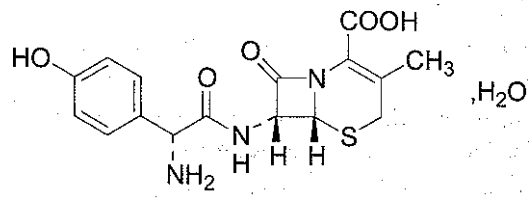
Calculate the content of $C_{15}H_{14}ClN_3O_4S$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cefaclor.

Cefadroxil

Cefadroxil Monohydrate



$C_{16}H_{17}N_3O_5S,H_2O$ Mol. Wt. 381.4

Cefadroxil is 7-[(*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid monohydrate.

Cefadroxil contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{16}H_{17}N_3O_5S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefadroxil IPRS* or with the reference spectrum of cefadroxil.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *1-tetradecane*, allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

Mobile phase. A mixture of 60 volumes of *0.1 M citric acid*, 40 volumes of *0.1 M disodium hydrogen phosphate* and 1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone*.

Test solution. A 0.2 per cent w/v solution of the substance under examination in *water*.

Reference solution (a). A 0.2 per cent w/v solution of *cefadroxil IPRS* in *water*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with a 0.2 per cent w/v solution of

ninhydrin in *ethanol*, dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

Tests

pH (2.4.24). 4.0 to 6.0, determined in a 5.0 per cent w/v suspension.

Specific optical rotation (2.4.22). +165° to +178°, determined in a 1.0 per cent w/v solution.

Related substances Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of mobile phase A.

Reference solution (a). Dissolve 10 mg of *D-α-(4-hydroxyphenyl)glycine IPRS (cefadroxil monohydrate impurity A IPRS)* in 10.0 ml of the mobile phase A.

Reference solution (b). Dissolve 10 mg of *7-aminodesacetoxycephalosporanic acid IPRS (cefadroxil monohydrate impurity B IPRS)* in 10.0 ml of the *phosphate buffer pH 7.0*.

Reference solution (c). Dilute 1.0 ml, each of, reference solution (a) and (b) to 100.0 ml with the mobile phase A.

Reference solution (d). Dissolve 10 mg, each of, *dimethylformamide* and *dimethylacetamide* in 10.0 ml of the mobile phase A. Dilute 1.0 of the solution to 100.0 ml with the mobile phase A.

Reference solution (e). Dilute 1.0 ml of the reference solution (c) to 25.0 ml with the mobile phase A.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *phosphate buffer pH 5.0*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
1	98	2
20	70	30
23	98	2
30	98	2

The relative retention time with respect to cefadroxil peak for dimethylformamide is about 0.4 and for diemthylacetamide is about 0.75.

Inject reference solution (c) and (e). The test is not valid unless the resolution between the peaks due to cefadroxil impurities A and B is not less than 5.0 in the chromatogram obtained with reference solution (c). In the chromatogram obtained with reference solution (e), signal- to- noise ratio for the second peak is not less than 10.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak due to cefadroxil impurity A is not more than the area of the first peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any secondary peak is not more than the area of the second peak in the chromatogram obtained with reference solution (c) (1.0 per cent). The sum of areas of all the secondary peaks is not more than 3 times the area of the second peak in the chromatogram obtained with reference solution (c) (3.0 per cent). Ignore any peak with an area less than 0.05 times the area of the second peak in the chromatogram obtained with reference solution (c) (0.05 per cent). Ignore the peaks due to dimethylformamide and dimethylacetamide.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method B.

Water (2.3.43). 4.2 to 6.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A freshly prepared 0.1 per cent w/v solution of the substance under examination in *phosphate buffer pH 5.0*.

Reference solution. A freshly prepared 0.1 per cent w/v solution of *cefadroxil IPRS* in *phosphate buffer pH 5.0*.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 96 volumes of *phosphate buffer pH 5.0* and 4 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{17}N_3O_5S$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Cefadroxil Capsules

Cefadroxil Capsules contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$.

Usual strength. The equivalent of 500 mg of anhydrous cefadroxil.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *1-tetradecane,* allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

Mobile phase. A mixture of 60 volumes of *0.1 M citric acid,* 40 volumes of *0.1 M disodium hydrogen phosphate* and 1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone.*

Test solution. Shake a quantity of the contents of a capsule with sufficient *water* to produce a solution containing 0.2 per cent w/v of Cefadroxil.

Reference solution (a). A 0.2 per cent w/v solution of *cefadroxil IPRS* in *water.*

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with a 0.2 per cent w/v solution of *ninhydrin* in *ethanol,* dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary, at the maximum at about 263 nm.

Calculate the content of $C_{16}H_{17}N_3O_5S$ in the medium from the absorbance obtained from a solution of known concentration of *cefadroxil IPRS.*

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{17}N_3O_5S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of content of capsules containing 0.5 g of anhydrous cefadroxil in 50 ml of the mobile phase, mix for 10 minutes and filter.

Reference solution (a). A 0.01 per cent w/v solution of *cefadroxil IPRS* in the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *D-α-(4-hydroxyphenyl) glycine IPRS (cefadroxil impurity A IPRS)* in the mobile phase.

Reference solution (c). A 0.01 per cent w/v solution of *7-aminodesacetoxycephalosporanic acid IPRS (cefadroxil impurity B IPRS)* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (10 µm) (Such as Bondapak C18),
- column temperature: 40°,
- mobile phase: add 20 volumes of *1M potassium hydroxide,* 4 volumes of *0.4 M tetrabutylammonium hydroxide* and 8 volumes of *methanol* in 160 volumes of *water* and dilute to 200 volumes with *water,* adjusted to pH 7.0 with *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 1500 theoretical plates and tailing factor is not more than 1.6 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a), (b), (c) and the test solution. Run the chromatogram 6 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to cefadroxil impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of peak corresponding to cefadroxil impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Water (2.3.43). Not more than 7.0 per cent, determined on 0.5 g of the mixed contents of 20 capsules.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.2 g of Cefadroxil, add sufficient *phosphate buffer pH 5.0,* shake for 30 minutes, dilute to 200.0 ml with the same solvent and filter.

Reference solution. A 0.1 per cent w/v solution of *cefadroxil IPRS* in *phosphate buffer pH 5.0.*

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 96 volumes of *phosphate buffer pH 5.0* and 4 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{17}N_3O_5S$ in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of anhydrous cefadroxil.

Cefadroxil Oral Suspension

Cefadroxil Mixture

Cefadroxil Oral Suspension is a mixture of Cefadroxil with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of *water* just before use.

Cefadroxil Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of $C_{16}H_{17}N_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cefadroxil.

Usual strengths. The equivalent of 125 mg and 250 mg of anhydrous cefadroxil per 5 ml after reconstitution.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *1-tetradecane*, allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

Mobile phase. A mixture of 60 volumes of *0.1 M citric acid*, 40 volumes of *0.1 M disodium hydrogen phosphate* and 1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone*.

Test solution. Dilute a suitable quantity of the freshly prepared suspension with water to obtain a solution containing 0.2 per cent w/v of cefadroxil. Filter the solution.

Reference solution (a). A 0.2 per cent w/v solution of *cefadroxil IPRS* in *water*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution After development, dry the plate in air, spray with a 0.2 per cent w/v solution of *ninhydrin* in *ethanol*, dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

Water (2.3.43). Not more than 2.0 per cent, determined on 1.0 g, using a mixture of 2 volumes of *carbon tetrachloride*, 2 volumes of *chloroform* and 1 volume of *methanol* in place of *methanol* in the titration vessel.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Tests

pH (2.4.24). 4.5 to 6.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the oral suspension containing about 0.1 g of Cefadroxil in 100.0 ml with mobile phase A, stir for 10 minutes and filter.

Reference solution (a). A 0.001 per cent w/v solution of *cefadroxil IPRS* in mobile phase A.

Reference solution (b). A 0.001 per cent w/v solution of *D-α-(4–hydroxyphenyl)glycine IPRS (cefadroxil impurity A IPRS)* in mobile phase A.

Reference solution (c). Dissolve 10 mg of *7- amino desacetoxycephalosphorinic acid IPRS* (*cefadroxil impurity B IPRS*) in 10.0 ml of *phosphate buffer pH 7.0* and dilute to 100.0 ml with mobile phase A. Dilute 5.0 ml of the solution to 50.0 ml with mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Lichrosorb RP–18),
- mobile phase: A. dissolve 5.44 g of *potassium dihydrogen orthophosphate* in 2000 ml of *water*, adjusted to pH 5.0 with *potassium hydroxide solution*, B. add 40 volumes of *acetonitrile* to 60 volumes of mobile phase A, adjusted to pH 5.0 with *orthophosphoric acid*,

- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
35	68	32
60	68	32
61	100	0
70	100	0

The retention time of cefadroxil is 14 to 20 minutes. If necessary, adjust the proportion of mobile phase A to mobile phase B to achieve the stated retention time.

Inject reference solution (a).The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 1.5.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to cefadroxil impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of the peak due to cefadroxil impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a weigh quantity of the suspension containing about 0.1g of cefadroxil to a 100-ml volumetric flask, add *phosphate buffer pH 5.0*, shake for 30 minutes, dilute to 100.0 ml with the same solvent and filter.

Reference solution. A 0.1 per cent w/v solution of *cefadroxil IPRS* in *phosphate buffer pH 5.0*.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 96 volumes of *phosphate buffer pH 5.0* and 4 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of $C_{16}H_{17}N_3O_5S$, weight in volume.

Repeat the procedure using a portion of the suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the quantity of active ingredient in terms of anhydrous cefadroxil.

Cefadroxil Tablets

Cefadroxil Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous cefadroxil, $C_{16}H_{17}N_3O_5S$.

Usual strengths. The equivalent of 500 mg and 1 g of anhydrous cefadroxil.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H* and impregnating the dry plate by placing it in a tank containing a shallow layer of about 1 cm of a mixture of 95 volumes of *n-hexane* and 5 volumes of *1-tetradecane*, allowing the solvent to ascend to the top, removing the plate and allowing the solvent to evaporate.

Mobile phase. A mixture of 60 volumes of *0.1 M citric acid*, 40 volumes of *0.1 M disodium hydrogen phosphate* and 1.5 volumes of a 6.66 per cent w/v solution of *ninhydrin* in *acetone*.

Test solution. Shake a quantity of the powdered tablets with sufficient water to produce a solution containing 0.2 per cent w/v of cefadroxil. Filter the solution.

Reference solution (a). A 0.2 per cent w/v solution of *cefadroxil IPRS* in *water*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 20 µl of each solution After development, dry the plate in air, spray with a 0.2 per cent w/v solution of *ninhydrin* in *ethanol*, dry at 110° for 10 minutes and examine. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 263 nm (2.4.7). Calculate the content of $C_{16}H_{17}N_3O_5S$ in the medium from the absorbance obtained from a solution of known concentration of *cefadroxil IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{17}N_3O_5S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing about 0.5 g of anhydrous cefadroxil in 50 ml of the mobile phase, mix for 10 minutes and filter.

Reference solution (a). A 0.01 per cent w/v solution of *cefadroxil IPRS* in the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *D-α-(4-hydroxyphenyl) glycine IPRS (cefadroxil impurity A IPRS)* in the mobile phase.

Reference solution (c). A 0.01 per cent w/v solution of *7-aminodesacetoxycephalosporanic acid IPRS (cefadroxil impurity B IPRS)* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (10 µm) (Such as µBondpak C18),
- column temperature: 40°,
- mobile phase: add 20 volumes of *1M potassium hydroxide*, 4 volumes of *0.4 M tetrabutylammonium hydroxide* and 8 volumes of *methanol* in 160 volumes of *water* and dilute to 200 volumes with *water*, adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Inject reference solution (a). The test is not valid unless the theoretical plates are not less than 1500 and tailing factor is not more than1.6 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a), (b), (c) and the test solution. For test solution, run the chromatogram 6 times the retention times of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to cefadroxil impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of peak corresponding to cefadroxil impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 8.0 per cent, determined on 0.5 g of the powdered tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the following solutions freshly.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.2 g of cefadroxil, dissolve in *phosphate buffer pH 5.0* by shaking for 30 minutes and dilute to 200.0 ml the same solvent. Filter the solution.

Reference solution. A 0.1 per cent w/v solution of *cefadroxil IPRS* in *phosphate buffer pH 5.0*.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 96 volumes of *phosphate buffer pH 5.0* and 4 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

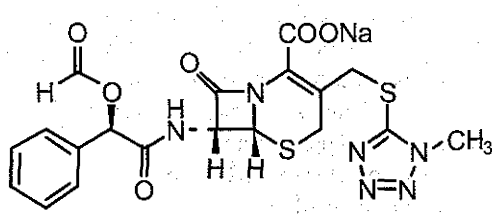
Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{17}N_3O_5S$ in the tablets.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of anhydrous cefadroxil.

Cefamandole Nafate



$C_{19}H_{17}N_6NaO_6S_2$ Mol. Wt. 512.5

Cefamandole Nafate is 7-D-mandelamido-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid.

Cefamandole Nafate containt is not less than 93.0 per cent and not more than 102.0 per cent of $C_{19}H_{17}N_6NaO_6S_2$, calculated on the anhydrous and sodium carbonate-free basis, for the sum of the content of cefamandole nafate, and cefamandole sodium expressed as cefamandole nafate.

Cefamandole Sodium containt is not more than 10.0 per cent of $C_{18}H_{17}N_6NaO_5S_2$, calculated on the anhydrous and sodium carbonate-free basis.

Sodium Carbonate contains not less than 4.8 per cent and not more than 6.4 per cent of Na_2CO_3.

Category. Antibacterial.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefamandole nafate IPRS* or with the reference spectrum of cefamandole nafate.

B. Gives the reactions of sodium salt (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and its absorbance at 475 nm (2.4.7) is not more than 0.03.

pH (2.4.24). 6.0 to 8.0, measured after 30 minutes, determined in solution A.

Specific optical rotation (2.4.24). −45.0° to −35.0°, determined in a 10.0 per cent w/v solution in *acetate buffer* pH 4.7 calculated on anhydrous and sodium carbonate-free basis.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Solvent mixture. 18 volumes of *acetonitrile* and 75 volumes of a 10 per cent v/v solution of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid.*

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture, then heat at 60 ° for 30 minutes.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 1 volume of *triethylamine phosphate buffer* prepared by dissolving 2.0 g of *sodium pentanesulphonate* in 350 ml of *water,* add 40 ml of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid* and dilute to 700 ml with *water,* and 2 volumes of *water,*

  B. a mixture of equal volumes of *triethylamine phosphate buffer*, *methanol* and *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
1	100	0
35	0	100
45	0	100
50	100	0

The relative retention time with reference to cefamandole nafate for cefamandole is about 0.8.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cefamandole and cefamandole nafate is not less than 5.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained in the with reference solution (b) (1.0 per cent). The sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent). Ignore any peaks with an area less than 0.1 time the area of the principle peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

2-Ethylhexanoic acid (2.3.51). Not more than 0.3 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Cefamandole Nafate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefamandole nafate.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *cefamandole nafate IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase, then heat at 60° for 30 minutes.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed, with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 10 per cent v/v solution of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 7.0. The relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{19}H_{17}N_6NaO_6S_2$ as the sum of the areas of the two peaks corresponding to cefamandole nafate and cefamandole sodium expressed as cefamandole nafate.

1 mg of $C_{18}H_{17}N_6NaO_5S_2$ is equivalent to 1.0578 mg of $C_{19}H_{17}N_6NaO_6S_2$.

Sodium carbonate. Dissolve 0.5 g of the substance under examination in 50 ml of *water*. Titrate with *0.1 M hydrochloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.0053 g of Na_2CO_3.

Storage. Store protected from light and moisture, if the substance is sterile, store in a sterile, air tight, tamper proof container.

Labelling. The label states that the substance contains sodium carbonate.

Cefamandole Injection

Cefamandole Nafate Injection

Cefamandole Injection is a sterile material consisting of Cefamandole Nafate with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requiremens for the Clarity of Solution and Particulate matter stated under Parental Preparations (Injections).

Usual strengths. The equivalent of 1 g; 2 g and 10 g of cefamandole.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefamandole Injection contains not less than 90.0 per cent and not more than 115.0 per cent of stated amount of cefamandole, $C_{18}H_{18}N_6O_5S_2$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G 254*.

Mobile phase. A mixture of 50 volumes of *ethyl acetate*, 20 volumes of *acetone*, 10 volumes of *glacial acetic acid* and 10 volumes of *water*.

Test solution. Disperse a quantity of injection containing 100 mg of Cefamandole in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution. A 1.0 per cent w/v solution of *cefamandole nafate IPRS* in the mobile phase.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8.0 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.0 to 8.0, determined in a 10.0 per cent w/v solution of cefamandole.

Bacterial endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefamandole.

Water (2.3.43). Not more than 3.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Disperse a quantity of the injection containing 50 mg of Cefamandole Nafate with the mobile phase and dilute to 100.0 ml of the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *cefamandole nafate IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase, then heat at 60° for 30 minutes.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 10 per cent v/v solution of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 7.0. The relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{19}H_{17}N_6NaO_6S_2$ as the sum of the areas of the two peaks corresponding to cefamandole nafate and cefamandole sodium expressed as cefamandole nafate.

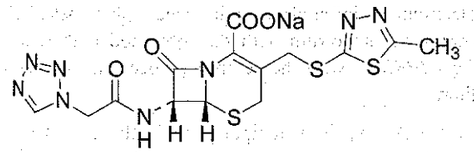
1 mg of $C_{19}H_{17}N_6NaO_6S_2$ (cefamandole nafate) is equivalent to 0.9024 mg of $C_{18}H_{18}N_6O_5S_2$ (cefamandole).

Storage. Store protected from moisture, in a sterile, tamper evident sealed container so as to exclude micro-organism, at a temperature not exceeding 30°.

Labelling. The label states the quantity of cefamandole nafade contained in the sealed container in terms of the equivalent amount of cefamandole.

Cefazolin Sodium

Cephazolin Sodium



$C_{14}H_{13}N_8NaO_4S_3$ Mol. Wt. 476.5

Cefazolin Sodium is sodium 7-[(1*H*)-tetrazol-1-ylacetamido]-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-3-cephem-4-carboxylate.

Cefazolin Sodium contains not less than 85.0 per cent and not more than 105.0 per cent of cefazolin $C_{14}H_{14}N_8O_4S_3$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefazolin sodium IPRS* or with the reference spectrum of cefazolin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reactions of sodium salts (2.2.1).

Tests

pH (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). –24.0° to –10.0°, determined in a 5.5 per cent w/v solution in *0.1 M sodium bicarbonate*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 20.0 ml of mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b). Dissolve 20 mg of the substance under examination in 10 ml of 0.2 per cent w/v solution of *sodium hydroxide*, allow to stand for 30 minutes. Dilute 1.0 ml of the solution to 20.0 ml with mobile phase A.

Chromatographic system
- a stainless steel column 12.5 cm x 4 mm packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 45°,
- mobile phase: A. a solution containing 1.45 per cent w/v of *disodium hydrogen phosphate* and 0.35 per cent w/v of *potassium dihydrogen phosphate*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
2	98	2
4	85	15
10	60	40
11.5	35	65
12	35	65
15	98	2
21	98	2

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefazolin and cefazolin impurity L is not less than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any

secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the sum of area of all the secondary peaks is not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

Assay. Determine by liquid chromatography (2.4.14).

Solution A. Prepared by dissolving 0.75 g of *salicylic acid* (internal standard) in 5 ml of *methanol* and diluting to 100.0 ml with *mixed phosphate buffer pH 7.0*.

Test solution. A 0.1 per cent w/v solution of the substance under examination in *mixed phosphate buffer pH 7.0*. to 5.0 ml of the solution add 5.0 ml of solution A and add sufficient volume of *mixed phosphate buffer pH 7.0* to produce 100.0 ml and mix.

Reference solution. A 0.1 per cent w/v solution of *cefazolin sodium IPRS* in *mixed phosphate buffer pH 7.0*. To 5.0 ml of the solution add 5.0 ml of solution A and add sufficient volume of *mixed phosphate buffer pH 7.0* to produce 100.0 ml and mix.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 9 volumes of *phosphate buffer pH 3.6* and 1 volume of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative retention times of salicylic acid and cefazolin are 0.7 and 1.0 respectively.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{14}N_8O_4S_3$.

Cefazolin Sodium intended for use in the manufacture of parenteral preparations complies with the following additional requirements.

Bacterial endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefazolin.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store in sterile containers, sealed so as to exclude micro-organisms protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the quantity of Cefazolin Sodium contained in the sealed container in terms of the equivalent amount of cefazolin.

Cefazolin Sodium Injection

Cefazolin Injection; Cephazolin Sodium Injection; Cephazolin Injection

Cefazolin Sodium Injection is a sterile material consisting of Cefazolin Sodium with or without excipients. It is filled in sealed containers.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Usual strengths. The equivalent of 125 mg; 250 mg; 500 mg; and 1 g of cefazolin.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefazolin Sodium Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of cefazolin, $C_{14}H_{14}N_8O_4S$.

Description. A white to off-white, crystalline powder; odourless.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefazolin sodium IPRS* or with the reference spectrum of cefazolin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reactions of sodium salts (2.2.1).

Tests

pH (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). −24.0° to −10.0°, determined in a 5.5 per cent w/v solution in *0.1 M sodium bicarbonate*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve an accurately weighed quantity of powder containing 0.25 g of cefazolin in 100.0 ml of mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b). Dissolve 20 mg of *cefazolin IPRS* in 10 ml of 0.2 per cent w/v solution of *sodium hydroxide*, allow to stand for 30 minutes. Dilute 1.0 ml of the solution to 20.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm packed with endcapped octadecylsilane bonded to porous silica (3 µm) (Such as Nucleosil C18),
- column temperature: 45°,
- mobile phase: A. a solution containing 1.45 per cent w/v of *disodium hydrogen phosphate* and 0.35 per cent w/v of *potassium dihydrogen phosphate*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
2	98	2
4	85	15
10	60	40
11.5	35	65
12	35	65
15	98	2
21	98	2

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cefazolin and cefazolin impurity L is not less than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the sum of areas of all the secondary peaks is not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.15 Endotoxin Unit per mg of cefazolin.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.15 g.

Assay. Determine by liquid chromatography (2.4.14).

Solution A. Prepare by dissolving 0.75 g of *salicylic acid* (internal standard) in 5 ml of *methanol* and diluting to 100.0 ml with *mixed phosphate buffer pH 7.0*.

Test solution. Determine the weight of the contents of 10 containers. Weigh accurately a quantity of the mixed contents of the 10 containers, dissolve in the *mixed phosphate buffer pH 7.0* and dilute to obtain a solution containing 0.1 per cent w/v of cefazolin. To 5.0 ml of the solution add 5.0 ml of solution A and add sufficient volume of *mixed phosphate buffer pH 7.0* to produce 100.0 ml and mix.

Reference solution. A 0.1 per cent w/v solution of *cefazolin sodium IPRS* in *mixed phosphate buffer pH 7.0*. To 5.0 ml of the solution add 5.0 ml of solution A and sufficient volume of *mixed phosphate buffer pH 7.0* to produce 100.0 ml and mix.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 9 volumes of *phosphate buffer pH 3.6* and 1 volume of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative retention times of salicylic acid and cefazolin are 0.7 and 1.0 respectively.

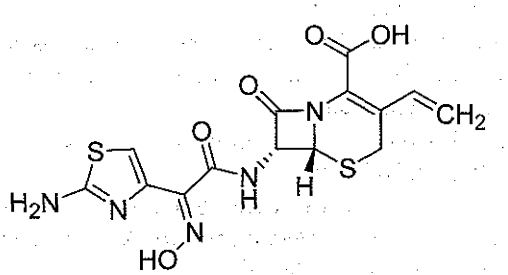
Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{14}N_8O_4S_3$ in the injection.

Storage. Store protected from moisture at a temperature not exceeding 30°. The constituted solution should be stored protected from light and used within 24 hours when stored at a temperature not exceeding 30° or within 4 days when stored between 2° and 8°.

Labelling. The label states the quantity of Cefazolin Sodium contained in the sealed container in terms of the equivalent amount of cefazolin.

Cefdinir



$C_{14}H_{13}N_5O_5S_2$ Mol.Wt. 395.4

Cefdinir is 5-thia 1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Cefdinir contains not less than 94.0 per cent and not more than 103.0 per cent of $C_{14}H_{13}N_5O_5S_2$, calculated on the anhydrous basis.

Category. Antibiotic.

Description. A white to light-yellow crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefdinir IPRS* or with the reference spectrum of cefdinir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Specific optical rotation (2.4.22). – 67.0° to – 61.0° at 20°, determined in a 1.0 per cent w/v solution in solvent mixture as described under Assay.

Related substances. A. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution (a). Dissolve 0.1 g of the substance under examination in the solvent mixture and dilute to 10.0 ml with solvent mixture.

Test solution (b). Dilute 3.0 ml of test solution (a) to 20.0 ml with solution A.

Reference solution (a). Dilute 1.0 ml of test solution (b) to 100.0 ml with solution A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with solution A.

Reference solution (c). A 0.15 per cent w/v solution of *cefdinir IPRS* and 0.01 per cent solution of *cefdinir impurity A IPRS* initially in the solvent mixture and then with solution A.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°
- mobile phase: A. a mixture of 1000 ml of solution A and add 0.4 ml of solution B,
  B. a mixture of 300 volumes of *acetonitrile*, 200 volumes of *methanol*, 500 volumes of a solution A and 0.4 volumes of solution B,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

Name	Relative retention time
Thiazolyacetyl glycine oxime[1]	0.10
Thiazolylacetyl glycine oxime acetal[2]	0.12
3-Methyl cefdinir[3]	0.74
Cefdinir related compound A (cefdinir open ring lactone a)[4,5]	0.85
Cefdinir related compound A (cefdinir open ring lactone b)[4,5]	0.93
Cefdinir related compound A (cefdinir open ring lactone c)[4,5]	1.11
Cefdinir related compound A (cefdinir open ring lactone d)[4,5]	1.14
Cefdinir lactone[6]	1.22
Cefdinir isoxazole analog[7]	1.36
E-Cefdinir[8]	1.51
Cefdinir decarboxy open ring lactone a[9,10]	1.61
Cefdinir decarboxy open ring lactone b[9,10]	1.64

[1]1N-[(Z)-2-(2-aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine,

[2](Z)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide,

[3](6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[4]2(*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido)]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-d][1,3]thiazin-2-yl]acetic acid,

[5]cefdinir related impurity A is a mixture of 4 isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported. The limit for the sum of the 4 isomers is 0.7 per cent,

[6](*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-{(3RS,5aR,6R)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl}acetamide,

[7](6*R*,7*R*)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[8](6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl-)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[9](*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-{[(2RS,5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-d][1,3]thiazin-2-yl]methyl}acetamide,

[a] Cefdinir decarboxy open ring lactone is a mixture of 2 isomers labeled cefdinir decarboxy open ring lactones a and b. The sum of the values is reported. The limit for sum of the 2 isomers is 0.5 per cent.116-demethylazithromycin.

Inject reference solution (a),(b) and (c). Cefdinir impurity A should produce four peaks.

The test is not valid unless the response ratio due to cefdinir peak obtained with reference solution (b) is between 7 per cent to 13 per cent of the response due to cefdinir peak obtained with reference solution (a) and the resolution between the peaks due cefdinir and third peak of cefdinir impurity A is not less than 1.5 obtained with reference solution (c).

Inject test solution (b). Run the chromatogram 1.8 times the retention time of the cefdinir peak.

In the chromatogram obtained with the test solution (b), the area of any peak corresponding to thiazolylacetyl glycine oxime and thiazolylacetyl glycine oxime acetal is not more than 0.5 per cent, the area of any peak corresponding to 3-methyl and thiazolylacetyl glycine oxime acetal is not more than 0.5 per cent, the area of any peak corresponding to cefdinir impurity A (cefdinir open ring lactone c) is not more than 0.7 per cent, the area of any peak corresponding to cefdinir lactone is not more than 0.5 per cent, the area of any peak corresponding to cefdinir isoxazole analog is not more than 0.5 per cent, the area of any peak corresponding to E-cefdinir is not more than 0.7 per cent, the area of any peak corresponding to cefdinir decarboxy open ring lactone b is not more than 0.5 per cent, the area of any secondary peak is not more (0.2 per cent) and the sum of the areas of all the secondary peaks is not 3.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 1.0 g complies with the limit test for the heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 2.0 per cent for anhydrous form. Not less than 4.0 per cent and not more than 8.5 per cent for hydrated form, by using a mixture of 67 volumes of *formamide* and 33 volumes of *methanol*.

Assay. Determine by liquid chromatography (2.4.14).

Solution A. Dilute *tetramethylammonium hydroxide solution* with *water* to obtain 0.1 per cent w/v solution, adjusted to pH 5.5 with *orthophosphoric acid*.

Solution B. A 3.72 per cent w/v solution of *disodium edetate*.

Solvent mixture. A mixture of 67 volumes of solution prepared by dissolving 14.2 g of *anhydrous dibasic sodium phosphate* in 1000 ml of *water* and 33 volumes of solution prepared by dissolving 13.6 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjusted to pH 7.0.

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with solvent mixture.

Reference solution (a). A solution containing 0.02 per cent w/v of *cefdinir IPRS* and 0.05 per cent w/v of *cefdinir impurity A IPRS* in the solvent mixture.

Reference solution (b). A 0.02 per cent w/v solution of the *cefdinir IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 60 volumes of *acetonitrile*,40 volumes of *methanol*, 900 volumes of a solution A and 0.4 volumes of solution B,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5 µl.

Inject reference solution (a) and (b). *Cefdinir impurity A IPRS* should produce four peaks.

The test is not valid unless the resolution between the peaks due to second peak of cefdinir impurity A and cefdinir is not less than 1.2 obtained with reference solution (a),the tailing factor is not more than 1.5 for cefdinir peak obtained with reference solution (a) and the relative standard deviation for replicate injections for cefdinir is not more than 1.0 per cent obtained with reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_{14}H_{13}N_5O_5S_2$.

Storage. Store protected from light and moisture.

Cefdinir Capsules

Cefdinir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefdinir, $C_{14}H_{13}N_5O_5S_2$.

Usual strength. 300 mg.

Identification

A. When examined in the range 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution of capsule powder containing cefdinir in the buffer solution prepared by dissolving 10.7 g of *dibasic sodium phosphate* and 3.4 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 7.0 ± 0.05 with *dilute orthophosphoric acid* or *sodium hydroxide solution*, shows an absorption maxima and minima at the same wavelength as the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.05 M phosphate buffer pH 6.8*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter having an average pore size not greater than 0.45 µm, rejecting the first few ml of the filtrate. Measure the absorbance of the resulting solution, suitably diluted if necessary with the dissolution medium, at the maximum at about 290 nm (2.4.7). Calculate the content of $C_{14}H_{13}N_5O_5S_2$ in the dissolution medium from the absorbance obtained from a solution of known concentration of *cefdinir IPRS* in dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{14}H_{13}N_5O_5S_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. A mixture of solutions containing 2:1 ratio of a solution prepared by dissolving 14.2 g of *anhydrous dibasic sodium phosphate* in 1000 ml of *water* and 13.6 g of *monobasic potassium phosphate* in 1000 ml of *water*, is maintained, adjusted to pH 7.0 ± 0.1.

Solution A. A 0.1 per cent v/v solution of *tetramethylammonium hydroxide solution (10 per cent)* in *water*, adjusted to pH 5.5 ± 0.1 with *dilute phosphoric acid*.

Solution B. A 3.72 per cent w/v solution of *sodium edetate* in *water*.

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing 300 mg of cefdinir and transfer to a 200-ml volumetric flask. Dissolve in 30 ml of the buffer solution, and dilute to volume with solution A to obtain 0.15 per cent w/v solution of cefdinir.

Reference solution (a). A 0.004 per cent w/v solution of *cefdinir related compound A IPRS (2R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2RS, 5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3] thiazin-2-yl]acetic acid* in solution A.

Reference solution (b). A 0.004 per cent w/v solution of *cefdinir related compound B IPRS (6R, 7R)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid* in solution A.

Reference solution (c). Transfer 37.5 mg of *cefdinir IPRS* to a 25-ml of volumetric flask. Add about 10 ml of the buffer solution. Add 5.0 ml each of reference solution (a) and reference solution (b), and dilute with solution A to volume.

Reference solution (d). A 0.075 per cent w/v solution of *cefdinir IPRS* in the buffer solution.

Reference solution (e). Dilute reference solution (d) to obtain a solution containing 0.0015 per cent w/v of *cefdinir IPRS* in solution A.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column. temperature: 40°,
- sample temperature: 4°,
- mobile phase: A. a mixture of 1000 volumes of solution A and 0.4 volume of solution B,
  B. a mixture of 150 volumes of *acetonitrile*, 100 volumes of *methanol*, 250 volumes of solution A and 0.2 volume of solution B,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
48	95	5

Name	Relative retention time	Correction factor
Thiazolylacetyl glycine oxime[1]	0.1	1.0
Thiazolylacetyl glycine oxime acetal[2]	0.13	1.0
Cefdinir sulfoxide[3]	0.36	1.0
Cefdinir thiazine analog[4]	0.46	1.47
3-Methyl cefdinir[5]	0.75	1.0
Cefdinir impurity 1[6]	0.77	1.0
Cefdinir related compound A (cefdinir open ring lactone a)[7-8]	0.85	1.54
Cefdinir related compound A (cefdinir open ring lactone b)[7-8]	0.94	1.54
Cefdinir related compound A (cefdinir open ring lactone c)[7-8]	1.11	1.54
Cefdinir related compound A (cefdinir open ring lactone d)[7-8]	1.14	1.54
7*S*-Cefdinir[9]	1.18	1.0
Cefdinir lactone[10]	1.23	1.0

Cefdinir related compound B[11]	1.28	1.0
Cefdinir isoxazole analog[12]	1.37	1.39
Cefdinir impurity2[6]	1.44	1.0
Cefdinir glyoxalic analog[13]	1.49	1.0
E-Cefdinir[14]	1.51	1.0
Cefdinir decarboxy openring lactone a[15-16]	1.62	1.0
Cefdinir decarboxy openring lactone b[15-16]	1.64	1.0
Cefdinir impurity 3[6]	1.82	1.0
Individual unidentified impurities	---	1.0
Total impurities	---	---

[1]*N*-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetyl]glycine,

[2](*Z*)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide,

[3](6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[4](*R*,*Z*)-2-{(*R*)-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido](carboxy)methyl}-5-ethylidene-5,6-dihydro-2*H* -1,3-thiazine-4-carboxylic acid,

[5](6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[6]Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities,

[7]Cefdinir related compound A is a mixture of four isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported. The limit for the sum of the four isomers is 2.5 per cent,

[8]2(*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H* -furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid,

[9](6*R*,7*S*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[10](*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-((3*RS*,5a*R*, 6*R*)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl)acetamide,

[11](6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[12](6*R*,7*R*)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[13](6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[14](6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,

[15]Cefdinir decarboxy open ring lactone is a mixture of two isomers labeled cefdinir decarboxy open ring lactone a and b. The sum of the values is reported. The limit for the sum of the two isomers is 1.0 per cent,

[16](*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-{[(2*RS*, 5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl] methyl}acetamide.

Inject reference solution (c) and (e). The test is not valid unless the resolution between the peaks due to cefdinir and cefdinir related compound A is not less than 1.5 in the chromatogram obtained with reference solution (c), the tailing factor for Cefdinir related compound B peak is not more than 1.5 in the chromatogram obtained with reference solution (c) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (e).

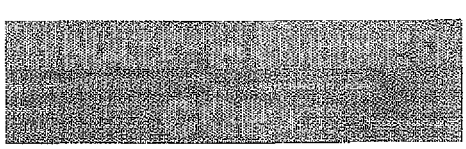
Inject reference solution (e) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to : thiazolylacetyl glycine oxime, thiazolylacetyl glycine oxime acetal, cefdinir isoxazole analog and cefdinir impurity 2 is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.5 per cent); cefdinir sulfoxide,7*S*-cefdinir, cefdinir related compound B, cefdinir glyoxalic analog and cefdinir impurity 3 is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent); cefdinir thiazine analog and 3- methyl cefdinir is not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.7 per cent); cefdinir lactone, cefdinir decarboxy open ring lactone a, cefdinir decarboxy open ring lactone b is not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (1.0 per cent); *E*-cefdinir is not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (e) (1.2 per cent); cefdinir impurity 1 is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.3 per cent); cefdinir related compound A (cefdinir open ring lactone a, b, c and d) is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (2.5 per cent) and the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent). The sum of areas of all the secondary peaks is not more than 5.0 times the area of the principal peak in the chromatogram obtained with reference solution (e) (5.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. A mixture prepared by dissolving 10.7 g of *dibasic sodium phosphate* and 3.4 g of *monobasic potassium phosphate* in 1000 ml of *water,* adjusted to pH 7.0 ± 0.05 with *dilute orthophosphoric acid* or *sodium hydroxide solution* before final dilution.

Solution A. A 0.7 per cent w/v solution of *citric acid monohydrate* in *water,* adjusted to pH 2.0 ± 0.05 with *orthophosphoric acid.*



Test solution. Weigh a quantity of the mixed contents of 20 capsules containing about 100 mg of Cefdinir, dissolve in the buffer solution by shaking mechanically, dilute to 100.0 ml with the buffer solution and filter. Dilute 5.0 ml of the solution to 100.0 ml with the buffer solution.

Reference solution (a). A solution containing 0.005 per cent w/v of *cefdinir IPRS* and 0.0175 per cent w/v of *m-hydroxybenzoic acid* in the buffer solution.

Reference solution (b). A 0.005 per cent w/v solution of *cefdinir IPRS* in the buffer solution.

Chromatographic system

- a stainless steel column 15 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 111 volumes of *methanol*, 28 volumes of *tetrahydrofuran* and 1000 volumes of solution A,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 15 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to cefdinir and *m*-hydroxybenzoic acid in the chromatogram obtained with reference solution (a) is not less than 3.0, the tailing factor for the peak due to cefdinir in the chromatogram obtained with reference solution (a) is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent for the peak due to cefdinir in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_{14}H_{13}N_5O_5S_2$ in the capsules.

Storage. Store protected from light, moisture and at a temperature not exceeding 25°.

Cefdinir Oral Suspension

Cefdinir Oral Suspension is a dry mixture consisting of Cefdinir with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of sealed container in the specified volume of *water* just before use.

Cefdinir Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of Cefdinir, $C_{14}H_{13}N_5O_5S_2$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of Cefdinir $C_{14}H_{13}N_5O_5S_2$.

Storage. Store protected from light and moisture.

Usual strength. Cefdinir 125 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

pH (2.4.24). 3.2 to 4.8.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution (a). 2 volumes of a solution prepared by dissolving 14.2 g of *anhydrous dibasic sodium phosphate* in 1000 ml *water* and 1 volume of a solution prepared by dissolving 13.6 g of *monobasic potassium phosphate* in 1000 ml *water*, to obtain a solution with a pH of 7.0.

Buffer solution (b). A 0.1per cent w/v solution of *tetramethylammonium hydroxide in water*, adjusted to pH 5.5 with *orthophosphoric acid*.

Buffer solution (c). A solution prepared by dissolving 37.2 g of *disodium edetate* in 1000 ml of *water*.

Test solution. Dissolve a quantity containing 0.15 g of cefdinir to a 100-ml volumetric flask in 30 ml of buffer solution (a), and dilute with buffer solution (b) to volume.

Reference solution (a). A 0.004 per cent w/v solution of *cefdinir related compound A IPRS* in buffer solution (b).

Reference solution (b). A 0.004 per cent w/v solution of *cefdinir related compound B IPRS* in buffer solution (a).

Reference solution (c). Weigh and transfer 37.5 mg of *cefdinir IPRS* to a 25 ml volumetric flask, and add about 10 ml of buffer solution (a). Add 5.0 ml each of reference solution (a) and reference solution (b), and dilute with buffer solution (b) to volume.

Reference solution (d). A 0.075 per cent w/v solution of *cefdinir IPRS* in buffer solution (a).

Reference solution (e). A 0.0015 per cent w/v solution of *cefdinir IPRS* from the reference solution (d) in buffer solution (b).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with *octadecyl silane* bonded to porous silica (5 µm),
- column temperature 40°,
- sample temperature 4°,
- mobile phase: A. a solution prepared by mixing 1000 ml of buffer solution (b) and 0.4 ml of buffer solution (c), B. a mixture of 150 volumes of *acetonitrile*, 100 volumes of *methanol*, 250 volumes of buffer solution (b) and 0.2 volume of buffer solution (c),

– a gradient programme using the conditions given below,
– flow rate: 1 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

Name	Relative retention time	Correction factor
Thiazolylacetyl glycine oxime[1]	0.10	—
Thiazolylacetyl glycine oxime acetal[2]	0.13	—
Cefdinir sulfoxide[3]	0.36	—
Cefdinir thiazine analog[4]	0.46	1.47
3-Methyl cefdinir[5]	0.75	—
Cefdinir impurity 1[6]	0.77	—
Cefdinir related compound A[7,8] (cefdinir open ring lactone a)	0.85	1.54
Cefdinir related compound A[7,8] (cefdinir open ring lactone b)	0.94	1.54
Cefdinir related compound A[7,8] (cefdinir open ring lactone c)	1.11	1.54
Cefdinir related compound A[7,8] (cefdinir open ring lactone d)	1.14	1.54
7S -Cefdinir[9]	1.18	—
Cefdinir lactone[10]	1.23	—
Cefdinir related compound B[11]	1.28	—
Cefdinir isoxazole analog[12]	1.37	1.39
Cefdinir impurity 2[6]	1.44	—
Cefdinir glyoxalic analog[13]	1.49	—
E-Cefdinir[14]	1.51	—
Cefdinir decarboxy open ring lactone a[15,16]	1.62	—
Cefdinir decarboxy open ring lactone b[15,16]	1.64	—
Cefdinir impurity 3[6]	1.82	—

[1]N-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.

[2](Z)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.

[3](6R ,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[4](*R*,*Z*)-2-{(*R*)-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido](carboxy)methyl}-5-ethylidene-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

[5](6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[6]Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities.

[7]Cefdinir related compound A is a mixture of four isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported; the limit for the sum of the four isomers is 3.3 per cent.

[8]2(*R*)-2-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2*RS*,5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-d][1,3] thiazin-2-yl]acetic acid.

[9](6*R*,7*S*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[10](*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-*N*-((3*RS*,5a*R*,6*R*)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d] [1,3]thiazin-6-yl)acetamide.

[11](6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[12](6*R*,7*R*)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[13](6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[14](6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino) acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[15]Cefdinir decarboxy open ring lactone is a mixture of two isomers labeled cefdinirdecarboxy open ring lactone a and b. The sum of the values is reported; the limit for the sum of the two isomers is 1.1 per cent.

[16](*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-{[(2*RS* 5*RS*)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1*H* -furo[3,4-d][1,3]thiazin-2-yl] methyl}acetamide.

Inject reference solution (c) and (e), The test is not valid unless the resolution between cefdinir and cefdinir related compound A is not less than 1.5, the tailing factor for the peak due to cefdinir related compound B in the chromatogram obtained with the reference solution (c) is not more than 1.5 and relative standard deviation for replicate injections is not more than 2.0 per cent with reference solution (e).

Inject reference solution (e) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to thiazolylacetyl glycine oxime, is not more than 0.5 times (0.5 per cent) and thiazolylacetyl glycine oxime acetal is not more than 0.6 times (0.6 per cent) the area of the principal peak in the chromatogram obtained with reference solution (e), the area of any peak corresponding to cefdinir sulfoxide, cefdinir impurity 1,7S-cefdinir, cefdinir related compound B, cefdinir impurity 2, cefdinir glyoxalic analog, and cefdinir impurity 3 is not more than 0.2 times the area of

the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent), the area of any peak corresponding to cefdinir thiazine analog is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.3 per cent), the area of any peak corresponding to cefdinir isoxazole analog is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.5 per cent), the area of any peak corresponding to 3-methyl cefdinir is not more than 0.7 times area of the principal peak in the chromatogram obtained with reference solution (e) (0.7 per cent), the area of any peak corresponding to cefdinir lactone is not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.8 per cent), the area of any peak corresponding to cefdinir decarboxy open ring lactone a and cefdinir decarboxy open ring lactone b is not more than 1.1 times the area of the principal peak in the chromatogram obtained with reference solution (e) (1.1 per cent), the area of any peak corresponding to E-cefdinir is not more than 1.4 times the area of the principal peak in the chromatogram obtained with reference solution (e) (1.4 per cent), the area of any peak corresponding to Cefdinir related compound A (cefdinir open ring lactone a), cefdinir related compound A (cefdinir open ring lactone b), cefdinir related compound A (cefdinir open ring lactone c), and cefdinir related compound A (cefdinir open ring lactone d is not more than 3.3 times the area of the principal peak in the chromatogram obtained with reference solution (e) (3.3 per cent). The area of any individual unidentified impurities is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent) and sum of the area of all the impurities is not more is not more than 6.2 times the area of the principal peak in the chromatogram obtained with reference solution (e) (6.2 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 10.7 g of *anhydrous dibasic sodium phosphate* and 3.4 g of *monobasic potassium phosphate* in 1000 ml of *water*. Adjusted to pH 7.0 with *dilute orthophosphoric acid* or *dilute sodium hydroxide* solution before final dilution.

Test solution. Disperse a quantity of the suspension containing 10 mg of cefdinir and transfer to a 200 ml volumetric flask, dilute with the buffer solution to the volume, filter, rejecting the first few ml of the filtrate and mix.

Reference solution (a). A solution containing 0.005 per cent w/v of *cefdinir IPRS* and 0.0175 per cent w/v of *m-hydroxybenzoic acid* in buffer solution.

Reference solution (b). 0.005 per cent solution of *cefdinir IPRS* in buffer solution.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecyl silane bonded to porous silica (4 µm),
- mobile phase: a mixture of 111 volumes of *methanol*, 28 volumes of *tetrahydrofuran* and 1000 volumes of a solution prepared by dissolving 7.0 g *citric acid monohydrate* in 1000 ml of *water*, adjusted to pH 2.0 with *orthophosphoric acid*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume:15 µl.

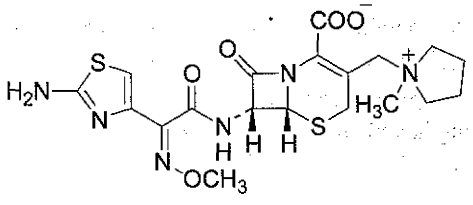
Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to cefdinir and m-hydroxybenzoic acid is not less than 3.0, the tailing factor for the peak due to cefdinir is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for the peak due to cefdinir.

Inject reference solution (b) and the test solution.

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{14}H_{13}N_5O_5S_2$, weight in volume.

Labelling. The label states (1) the quantity of active ingredient in terms of the equivalent amount of cefdinir; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

Cefepime Hydrochloride



, HCl, H_2O

$C_{19}H_{25}ClN_6O_5S_2,HCl,H_2O$ Mol. wt. 571.5

Cefepime Hydrochloride is 1-[[(6*R*, 7*R*)-7-[[(2*Z*)-(2-aminothiazol-4-yl) (methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride monohydrochloride monohydrate.

Cefepime Hydrochloride contains not less than 825 µg and not more than 911 µg of cefepime, $C_{19}H_{24}N_6O_5S_2$, per mg, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white to off-white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefepime*

hydrochloride IPRS or with the reference spectrum of cefepime hydrochloride.

Tests

Appearance of solution. A 10 per cent w/v solution is clear (2.4.1) and is not more intensely coloured than reference solution YS3 (2.4.1).

***N*-methylpyrrolidine.** Not more than 0.3 per cent.

Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *0.01 M nitric acid.*

Reference solution (a). Dilute 0.15 g of *N-methylpyrrolidine* to 100.0 ml with *water* and mix. Dilute 2.0 ml of the solution to 100.0 ml with *0.01 M nitric acid.*

Reference solution (b). Dilute 0.15 g of *pyrrolidine* to 100.0 ml with *water* and mix. Dilute 2.0 ml of the solution to 100.0 ml with *0.01 M nitric acid.* Mix 5.0 ml of the solution with 5.0 ml of reference solution (a).

Chromatographic system

- a stainless steel column 5 cm × 4.6 mm, packed with a strong cation exchange resin (5 µm),
- mobile phase: a mixture of 100 volumes of *0.01 M nitric acid* and 1 volume of *acetonitrile*,
- flow rate: 1 ml per minute,
- conductivity detector,
- injection volume: 100 µl.

Inject reference solution (a) and (b). In the chromatogram obtained with reference solution (a) the tailing factor for the peak due to N-methylpyrrolidine is not more than 2.5 and the relative standard deviation for replicate injections of reference solution (a) is not more than 5.0 per cent. In the chromatogram obtained with reference solution (b) the peak to valley ratio between the peaks due to pyrrolidine and N-methylpyrrolidine is not less than 3.

Inject the test solution. Continue the chromatography for 1.1 times the retention time of cefepime (about 50 minutes), eluting as a broadened peak.

Calculate the content of N-methylpyrrolidine.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 70 mg of the substance under examination in mobile phase A, stir with the aid of ultrasound for about 5 minutes and add sufficient mobile phase to produce 50.0 ml

Reference solution (a). A 0.14 per cent w/v solution of *cefepime hydrochloride IPRS* in mobile phase A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with mobile phase A. Dilute 2.0 ml of the solution to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile* and 90 volumes of a 0.068 per cent w/v solution of *potassium dihydrogen phosphate,* adjusted to pH 5.0 with *dilute phosphoric acid,*

  B. a mixture of equal volumes of *acetonitrile* and a 0.068 per cent w/v solution of *potassium dihydrogen phosphate,* adjusted to pH 5.0 with *dilute orthophosphoric acid,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	100	0
10	100	0
30	50	50
35	50	50
36	100	0
45	100	0

The relative retention times with reference to cefepime are 2.5 for [(6*R*,7*R*)-7-[[(2*E*)-(2-aminothiazol-4-yl)(methoxyimino) acetyl]amino]-3-[(1-methylpyrrolidinio) methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate] (cefepime impurity A) and about 4.1 for [(6*R*,7*R*)-7-[[(2*Z*)-(2-aminothiazol-4-yl) (methoxyimino)acetyl]amino]thiazol-4-yl](methoxyimino) acetyl]amino]-3-[(1-methylpyrrolidinio) methyl-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate] (cefepime impurity B).

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 1.5.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak due to cefepime impurity A is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.3 per cent), the area of any secondary peak due to cefepime impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (1.0 per cent). Ignore any peak

with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 3.0 per cent to 4.5 per cent, determined on 0.4 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 70 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution. A 0.14 per cent w/v solution of *cefepime hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 94 volumes of a solution prepared by dissolving 5.76 g of *sodium 1-pentanesulfonate* in 2000 ml of *water*, adjusted to pH 3.4 with *glacial acetic acid* and then pH 4.0 with *potassium hydroxide*, and 6 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0. The column efficiency is not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{24}N_6O_5S_2$.

Cefepime Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.04 Endotoxin Unit per mg.

Cefepime Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Cefepime Injection

Cefepime Hydrochloride Injection

Cefepime Injection is sterile mixture of Cefepime Hydrochloride and Arginine. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period not exceeding 7 days, recommended by the manufacturer provided the solution is stored in a refrigerator (2° to 8°).

Cefepime Injection contains Cefepime Hydrochloride equivalent to not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of cefepime, $C_{19}H_{24}N_6O_5S_2$.

Usual strengths. 250 mg; 500 mg; 1 g.

Description. A white to pale yellow powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for injection) and with the following requirements.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 70 volumes of *n-propyl alcohol*, 50 volumes of *water* and 40 volumes of *strong ammonia solution*.

Test solution. Weigh accurately a quantity equivalent to about 0.4 g of cefepime and dissolve in sufficient *water* to produce 10 ml.

Reference solution. Weigh accurately about 0.2 g of *L-arginine IPRS* and dissolve in 10 ml of *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 100° until the ammonia disappears completely. Spray the plate with a 0.2 per cent w/v solution of *ninhydrin* in a mixture of 95 volumes of *butyl alcohol* and 5 volumes of *2 M acetic acid*. Heat the plate at 105° for 15 minutes. Cool and examine in daylight. The dark red spot due to arginine in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

B. In the Assay the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.0 to 6.0, determined in a solution containing about 100 mg of cefepime per ml.

***N*-methylpyrrolidine**. Not more than 1.0 per cent.

Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve a quantity of injection containing 1.0 g of cefepime in *0.01 M nitric acid* and dilute to 100.0 ml with *0.01 M nitric acid*.

Reference solution (a). Dilute 0.5 g of *N-methylpyrrolidine* to 100.0 ml with *water* and mix. Dilute 2.0 ml of the solution to 100.0 ml with *0.01 M nitric acid*.

Reference solution (b). Dilute 0.15 g of *pyrrolidine* to 100.0 ml with *water* and mix. Dilute 2.0 ml of the solution to 100.0 ml with *0.01 M nitric acid*. Mix 5.0 ml of the solution with 5.0 ml of reference solution (a).

Chromatographic system

- a stainless steel column 5 cm × 4.6 mm, packed with a strong cation exchange resin (5 µm),
- mobile phase: a mixture of 100 volumes of *0.01 M nitric acid* and 1 volume of *acetonitrile*,
- flow rate: 1 ml per minute,
- conductivity detector,
- injection volume: 100 µl.

Inject reference solution (a) and (b). In the chromatogram obtained with reference solution (a) the tailing factor for the peak due to N-methylpyrrolidine is not more than 2.5 and the relative standard deviation for replicate injections of reference solution (a) is not more than 5.0 per cent. In the chromatogram obtained with reference solution (b) the peak to valley ratio between the peaks due to pyrrolidine and *N*-methylpyrrolidine is not less than 3.

Inject the test solution. Continue the chromatography for 1.1 times the retention time of cefepime (about 50 minutes), eluting as a broadened peak.

Calculate the content of N-methylpyrrolidine.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Prepare the solutions immediately before use.

Test solution. Dissolve a quantity of injection containing 70 mg of cefepime in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a). A 0.17 per cent w/v solution of *cefepime hydrochloride IPRS* in mobile phase A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with mobile phase A. Dilute 2.0 ml of the solution to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile* and 90 volumes of 0.068 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 5.0 with *dilute orthophosphoric acid*,

  B. a mixture of equal volumes of *acetonitrile* and a 0.068 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 5.0 with *dilute orthophosphoric acid*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	100	0
10	100	0
30	50	50
35	50	50
36	100	0
45	100	0

The relative retention times with reference to cefepime are 2.5 for (6*R*,7*R*)-7-[[(2*E*)-(2-aminothiazol-4-yl)(methoxyimino) acetyl]amino]-3-[(1-methylpyrrolidinio)methyl]-8-oxo-5-thia -1-azabicyclo[4.2.0] oct-2-ene-2-carboxylate (cefepime impurity A) and about 4.1 for (6*R*,7*R*)-7-[[(2*Z*)-(2-aminothiazol-4-yl) (methoxyimino) acetyl]amino]-4-yl](methoxyimino acetyl] amino]-4-yl] (methoxyimino)acetyl]amino]-3-[(1-methyl-pyrrolidinio) methyl]-8-oxo-5-thia-1-azabi-cyclo[4.2.0]oct-2-ene-2-carboxylate (cefepime impurity B).

Inject the reference solution (a). The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 1.5.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to cefepime impurity A is not more than 2.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak due to cefepime impurity B is not more than 2.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 7.5 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (1.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Bacterial Endotoxins (2.2.3). Not more than 0.06 Endotoxin Unit per mg of cefepime.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.5 g

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 10 containers. Dissolve a quantity of the mixed contents containing 70 mg of cefepime in 50 ml of the mobile phase.

Reference solution. A 0.17 per cent w/v solution of *cefepime hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 94 volumes of a solution prepared by dissolving 5.76 g of *sodium 1-pentanesulphonate* in 2000 ml of *water*, adjusted to pH 3.4 with *glacial acetic acid* and then pH 4.0 with *potassium hydroxide*, and 6 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

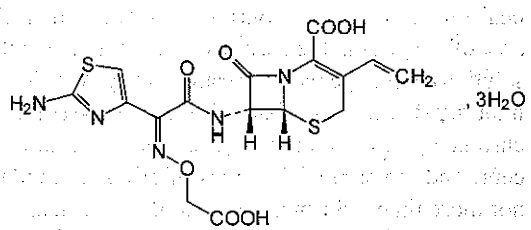
Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{24}N_6O_5S_2$ in the injection.

Storage. Store protected in sterile containers so as to exclude micro-organisms, at a temperature not exceeding 30°. Protect from light.

Labelling. The label states the strength in terms of the equivalent amount of cefepime.

Cefixime



$C_{16}H_{15}N_5O_7S_2, 3H_2O$ Mol. Wt. 507.5

Cefixime is (6*R*,7*R*)-7-[[(2*Z*)-2-(2-aminothiazol-4-yl)-[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trihydrate.

Cefixime contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{16}H_{15}N_5O_7S_2$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white to light yellow, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefixime IPRS*. If the spectra obtained show differences, dissolve the substance under examination and the reference substance separately in *methanol*, evaporate to dryness and record new spectra using the residues or with the reference spectrum of cefixime.

Tests

pH (2.4.24). 2.6 to 4.1, determined in a 5.0 per cent w/v suspension in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay.

Inject reference solution (b) and the test solution and continue the chromatography for 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak, other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks, other than the principal peak, is not greater than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent). Ignore any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). 9.0 per cent to 12.0 per cent, determined on 0.20 g.

Assay. Determine by liquid chromatography (2.4.14).

Phosphate buffer pH 7.0. Dissolve 7.1 g *dibasic sodium phosphate* in *water* and dilute to 500 ml with *water*. Adjust the pH of the solution to 7.0 with *monobasic potassium phosphate solution*.

Monobasic potassium phosphate solution. Dissolve 6.8 g of *monobasic potassium phosphate* in *water* and dilute to 500 ml with *water*.

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of *phosphate buffer pH 7.0*.

Reference solution (a). A 0.02 per cent w/v solution of *cefixime IPRS* in *phosphate buffer pH 7.0*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *phosphate buffer pH 7.0.*

Reference solution (c). Dissolve 10 mg of *cefixime IPRS* in 10 ml of *water*. Heat on a water-bath for 45 minutes. Cool and inject immediately.

Chromatographic system
- a stainless steel column 12.5 cm × 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a *tetrabutylammoniumhydroxide solution* prepared by diluting 25 ml of *0.4 M tetrabutylammoniumhydroxide solution* to 1000 ml with *water* and adjusted to pH 6.5 with *1.5 M ortho-phosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (c). The relative retention times are about 0.9 for cefixime *E*-isomer and 1.0 for cefixime and the resolution between cefixime and cefixime *E*-isomer is not less than 2.0.

Inject reference solution (a). The column efficiency is not less than 4000 theoretical plates, the tailing factor is not less than 0.9 and not more than 2.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{15}N_5O_7S_2$.

Storage. Store protected from light.

Cefixime Oral Suspension

Cefixime Oral Suspension is a mixture consisting of Cefixime with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Cefixime Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefixime $C_{16}H_{15}N_5O_7S_2$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cefixime $C_{16}H_{15}N_5O_7S_2$.

Usual strengths. 100 mg per 5 ml; 200 mg per 5 ml; 400 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Water (2.3.43). Not more than 2.0 per cent.

The constituted suspension complies with the tests stated under Oral Liquids and with the following tests.

Tests

pH (2.4.24). 2.5 to 4.5.

Assay. Determine by liquid chromatography (2.4.14).

Phosphate buffer pH 7.0. Dissolve 7.1 g *dibasic sodium phosphate* in *water* and dilute to 500 ml with *water*. Adjusted to pH 7.0 with *monobasic potassium phosphate solution*.

Monobasic potassium phosphate solution. Dissolve 6.8 g of *monobasic potassium phosphate* in *water* and dilute to 500 ml with *water*.

Test solution. Dilute an accurately weighed quantity of the oral suspension with *phosphate buffer pH 7.0* to obtain a solution having a concentration of 0.2 mg of cefixime per ml.

Reference solution (a). A 0.02 per cent w/v solution of *cefixime IPRS* in *phosphate buffer pH 7.0.*

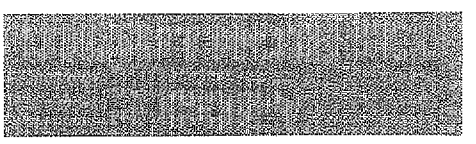
Reference solution (b). Dissolve 10 mg of *cefixime IPRS* in 10 ml of *water*. Heat the solution at 95° for 45 minutes. Cool and inject immediately.

Chromatographic system
- a stainless steel column 12.5 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature: 40°,
- mobile phase: a mixture of 30 volumes of *tetrabutylammonium hydroxide solution* prepared by diluting 25 ml of *0.4 M tetrabutylammonium hydroxide solution* to 1000 ml with *water* and adjusted to pH 6.5 with *1.5 M orthophosphoric acid*, and 10 volumes of *acetonitrile*,
- flow rate adjusted so that the retention time of cefixime is about 10 minutes,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (b).The relative retention times are about 0.9 for cefixime *E*-isomer and 1.0 for cefixime and the resolution between cefixime and cefixime *E*-isomer is not less than 2.0.

Inject reference solution (a). The column efficiency is not less than 4000 theoretical plates, the tailing factor is not less than 0.9 and not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.



Determine the weight per ml (2.4.29) of the oral suspension and calculate the content of $C_{16}H_{15}N_5O_7S_2$ weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at a temperature not exceeding 30°, for the period stated on the label. Calculate the content of $C_{16}H_{15}N_5O_7S_2$ weight in volume.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states (1) the quantity of active ingredient in terms of the equivalent amount of cefixime; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

Cefixime Dispersible Tablets

Cefixime Dispersible Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefixime, $C_{16}H_{15}N_5O_7S_2$.

Usual strengths. The equivalent of 50 mg ,100 mg and 200 mg of cefixime.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 10.0 per cent .

Assay. Determine by liquid chromatography (2.4.14).

Phosphate buffer pH 7.0. Dissolve 7.1 g *anhydrous dibasic sodium phosphate* in *water* and dilute to 500 ml with *water*. Adjust the pH of the solution to 7.0 with *monobasic potassium phosphate solution*.

Monobasic potassium phosphate solution. Dissolve 6.8 g of *monobasic potassium phosphate* in *water* and dilute to 500 ml with *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.4 g of Cefixime, disperse in 100.0 ml of *phosphate buffer pH 7.0*, mix with the aid of ultrasound and centrifuge. Dilute 5.0 ml of the clear supernatant to 100.0 ml with *phosphate buffer pH 7.0*.

Reference solution (a). A 0.022 per cent w/v solution of *cefixime IPRS* in *phosphate buffer pH 7.0*.

Reference solution (b). Dissolve 10 mg of *cefixime IPRS* in 10 ml of *water*. Heat the solution at 95° for 45 minutes. Cool and inject immediately.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 30 volumes of *tetrabutylammonium hydroxide solution* prepared by diluting 25 ml of *0.4 M tetrabutylammonium hydroxide solution* to 1000 ml with *water*, adjusted to pH 6.5 with *dilute orthophosphoric acid*, and 10 volumes of *acetonitrile*,
- flow rate adjusted so that the retention time of cefixime is about 10 minutes,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (b).The relative retention times are about 0.9 for cefixime *E*-isomer and 1.0 for cefixime and the resolution between cefixime and cefixime *E*-isomer is not less than 2.0.

Inject reference solution (a). The column efficiency is not less than 2000 theoretical plates, the tailing factor is not less than 0.9 and not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{15}N_5O_7S_2$ in the tablets.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) the strength in terms of the equivalent amount of cefixime; (2) that the tablets should be dispersed in *water* immediately before use.

Cefixime Tablets

Cefixime Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefixime, $C_{16}H_{15}N_5O_7S_2$.

Usual strengths. 50 mg; 100 mg; 200 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.05 M potassium phosphate buffer pH 7.2*, prepared by dissolving 6.8 g of *monobasic potassium*

phosphate in 1000 ml of *water*, adjusted to pH 7.2 with *1 M sodium hydroxide*,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 288 nm (2.4.7). Calculate the content of $C_{16}H_{15}N_5O_7S_2$ in the medium from the absorbance obtained from a solution of known concentration of *cefixime IPRS* in the same medium.

NOTE — A small amount of methanol not exceeding 0.1 per cent of the total volume may be used to dissolve cefixime and the solution may be mixed with the aid of ultrasound to assure complete dissolution.

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{15}N_5O_7S_2$.

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 10.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Phosphate buffer pH 7.0. Dissolve 7.1 g *dibasic sodium phosphate* in *water* and dilute to 500 ml with *water*. Adjust the pH of the solution to 7.0 with *monobasic potassium phosphate solution*.

Monobasic potassium phosphate solution. Dissolve 6.8 g of *monobasic potassium phosphate* in *water* and dilute to 500 ml with *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.4 g of cefixime, disperse in 100.0 ml of *phosphate buffer pH 7.0*, mix with the aid of ultrasound and centrifuge. Dilute 5.0 ml of the clear supernatant to 100.0 ml with *phosphate buffer pH 7.0*.

Reference solution (a). A 0.02 per cent w/v solution of *cefixime IPRS* in *phosphate buffer pH 7.0*.

Reference solution (b). Dissolve 10 mg of *cefixime IPRS* in 10 ml of *water*. Heat the solution at 95° for 45 minutes. Cool and inject immediately.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature: 40°,
- mobile phase: a mixture of 30 volumes of *tetrabutylammonium hydroxide solution* prepared by diluting 25 ml of *0.4 M tetrabutylammonium hydroxide solution* to 1000 ml with *water* and adjusted to pH 6.5 with *1.5 M orthophosphoric acid*, and 10 volumes of *acetonitrile*,
- flow rate adjusted so that the retention time of cefixime is about 10 minutes,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (b).The relative retention times are about 0.9 for cefixime *E*-isomer and 1.0 for cefixime and the resolution between cefixime and cefixime *E*-isomer is not less than 2.0.

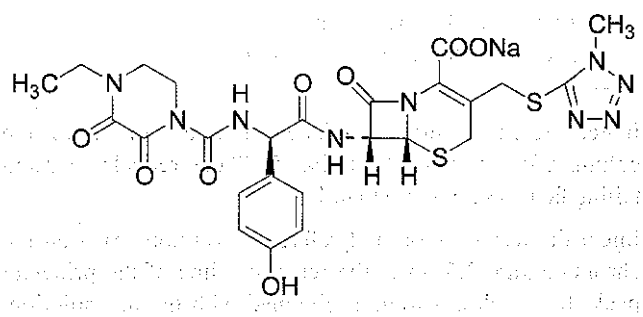
Inject reference solution (a). The column efficiency is not less than 2000 theoretical plates, the tailing factor is not less than 0.9 and not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{15}N_5O_7S_2$ in the tablets.

Storage. Store protected from moisture.

Cefoperazone Sodium



$C_{25}H_{26}N_9NaO_8S_2$ Mol Wt. 667.7

Cefoperazone sodium is sodium salt of 7-D-(-)-α-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-α-(4-hydroxyphenyl) acetamido-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl -3-cephem-4-carboxylic acid.

Cefoperazone Sodium contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{25}H_{26}N_9NaO_8S_2$, calculated on the anhydrous and solvent-free basis.

Category. Antibacterial.

Description. A white or almost white crystalline powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 25.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE —Use freshly prepared solutions.

Test solution. Dissolve 25 mg of the substance under examination in 50 ml of the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *cefoperazone sodium IPRS* in the mobile phase.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 884 volumes of *water*, 110 volumes of *acetonitrile*, 3.5 volumes of a 6 per cent w/v solution of *acetic acid*, 2.5 volumes of *triethylammonium acetate* solution prepared by diluting 14 ml of *triethylamine* and 5.7 ml of *glacial acetic acid* to 100 ml with *water*,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and tailing factor is not more than 1.6.

Inject the test solution and reference solution (b). Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent), the sum of areas of all the secondary peaks is not more than 4.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Acetone. Not more than 2.0 per cent.

Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.5 g of the substance under examination in 10.0 ml of *water*.

Reference solution. Dissolve 0.35 g of *acetone* in 100.0 ml of *water*. Dilute 10.0 ml of the solution to 100.0 ml with *water*.

Chromatographic system
- a fused-silica capillary or wide-bore column 30 m long and 0.32 mm or 0.53 mm, coated with *macrogol 20 000* (0.25 µm),
- temperature:
  column. 40°,
  inlet port. 140°,
- a flame ionisation detector at 250°,
- split ratio 1:5 with a linear velocity of about 35cm per second of the carrier gas.

Head-space injection conditions:
- equilibration time: 15 minutes,
- transfer-line temperature: 110°,

Inject 1 µl of the reference solution and the test solution.

Calculate the content of acetone.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *cefoperazone sodium IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 884 volumes of *water*, 110 volumes of *acetonitrile*, 3.5 volumes of a 6 per cent w/v solution of *acetic acid* and 2.5 volumes of a solution prepared by dissolving 14 ml of *triethylamine* and 5.7 ml of *glacial acetic acid* in 100 ml of *water*, and mixed,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is at most 1.6 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{26}N_9NaO_8S_2$.

Cefoperazone Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefoperazone sodium.

Cefoperazone Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture.

Labelling. The label states whether it is intended for use in the manufacture of parenteral preparations.

Cefoperazone Injection

Cefoperazone Sodium Injection

Cefoperazone Injection is a sterile material consisting of Cefoperazone Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Usual strengths. The equivalent of 250 mg; 500 mg; 1 g and 2 g of cefoperazone.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefoperazone Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefoperazone, $C_{25}H_{27}N_9O_8S_2$.

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. It gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 25.0 per cent w/v solution.

Bacterial endotoxins (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefoperazone.

Water (2.3.43). Not more than 5.0 per cent, except that where it is in the freeze-dried form, the limit is not more than 2.0 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 10 containers. Dissolve a quantity of the mixed contents containing 25 mg of cefoperazone, dissolve in the mobile phase and dilute to 250.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *cefoperazone sodium IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 884 volumes of *water*, 110 volumes of *acetonitrile*, 3.5 volumes of a 6 per cent w/v solution of *acetic acid* and 2.5 volumes of a solution prepared by dissolving 14 ml of *triethylamine* and 5.7 ml of *glacial acetic acid* in 100 ml of *water*, and mixed,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 5000, the tailing factor is at most 1.6 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

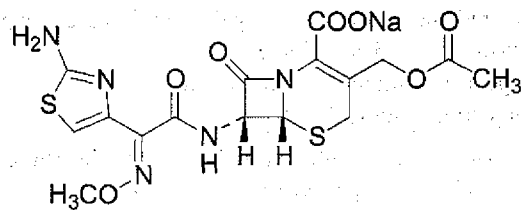
Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{27}N_9O_8S_2$ in the injection.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states the quantity of Cefoperazone Sodium contained in the sealed container in terms of the equivalent amount of cefoperazone.

Cefotaxime Sodium



$C_{16}H_{16}N_5NaO_7S_2$ Mol. Wt. 477.4

Cefotaxime Sodium is sodium (7*R*)-3-acetoxymethyl-7-[(*Z*)-2-(2-aminothiazol-yl)-2-(methoxyimino) acetamido]-3-cepham-4-carboxylate.

Cefotaxime Sodium contains the equivalent of not less than 91.6 per cent and not more than 96.4 per cent of cefotaxime, $C_{16}H_{17}N_5O_7S_2$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. An off-white to pale yellow, crystalline powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. It gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 10.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. 14 volumes of mobile phase B and 86 volumes of mobile phase A.

Test solution. Dissolve 40 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Add 1.0 ml of *dilute hydrochloric acid* to 4.0 ml of the test solution. Heat the solution at 40° for 2 hours. Add 5.0 ml of *buffer solution pH 6.6* and 1.0 ml of *dilute sodium hydroxide solution.*

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: A. a 0.71 per cent w/v solution of *disodium hydrogen phosphate,* adjusted to pH 6.25 with *orthophosphoric acid,*
  B. *methanol,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	86	14
7	86	14
9	82	18
16	82	18
45	60	40
50	60	40
55	86	14
60	86	14

The retention time of cefotaxime is about 13 minutes and the relative retention time with reference to cefotaxime for cefotaxime impurity A is about 0.6.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to deacetylcefotaxime lactone (cefotaxime impurity A) and cefotaxime is not less than 3.5 and the tailing factor of the principal peak is not more than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (2.3.43). Not more than 3.0 per cent, determined on 0.15 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A 0.01 per cent w/v solution of the substance under examination in *water.*

Reference solution. A 0.01 per cent w/v solution of *cefotaxime sodium IPRS* in *water.*

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a solution prepared by dissolving 60 mg of *potassium dihydrogen phosphate* and 1.2 g of *disodium hydrogen phosphate* in 1000 ml of *water* and mixing with 120 ml of *methanol,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{17}N_5O_7S_2$.

Cefotaxime Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefotaxime.

Cefotaxime Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture in tamper-evident containers.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Cefotaxime Sodium Injection

Cefotaxime Injection

Cefotaxime Sodium Injection is a sterile material consisting of Cefotaxime Sodium with or without excipients. It is filled in sealed containers.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefotaxime Sodium Injection contains a quantity of Cefotaxime Sodium equivalent to not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of cefotaxime, $C_{16}H_{17}N_5O_7S_2$.

Usual strengths. The equivalent of 250 mg, 1 g and 2 g of cefotaxime.

Description. An off-white to pale yellow, crystalline powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 10.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the injection containing about 0.1 g of Cefotaxime in 100.0 ml of the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: dissolve 3.5 g of *potassium dihydrogen orthophosphate* and 11.6 g of *disodium hydrogen orthophosphate* in 1000 ml of *water* and add 375 ml of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 10 µl.

Inject the reference solution and the test solution. Run the chromatogram 8 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (4.0 per cent).

Water (2.3.43). Not more than 3.0 per cent, determined on 0.15 g.

Bacterial endotoxins (2.2.3). Not more than 0.20 Endotoxin Unit per mg of cefotaxime.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 10 containers. Dissolve a quantity of the mixed contents containing 0.01 per cent w/v of cefotaxime.

Reference solution. A 0.01 per cent w/v solution of *cefotaxime sodium IPRS* in *water.*

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a solution prepared by dissolving 60 mg of *potassium dihydrogen phosphate* and 1.2 g of *disodium hydrogen phosphate* in 1000 ml of *water* and mixing with 120 ml of *methanol,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

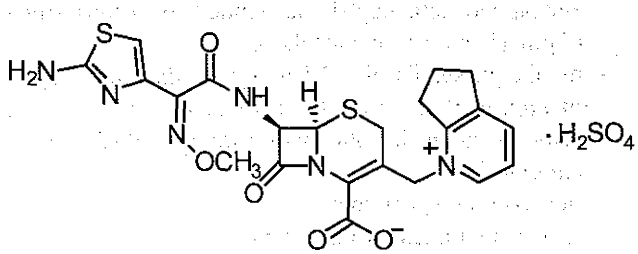
Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{17}N_5O_7S_2$ in the injection.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of cefotaxime.

Cefpirome Sulphate



$C_{22}H_{22}N_6O_5S_2, H_2SO_4$ Mol. Wt. 612.66

Cefpirome Sulphate is –[[(6R,7R)-7-[[(2Z)-(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-6,7-dihydro-5*H*-cyclopenta[*b*]pyrindinium sulphate.

Cefpirome Sulphate is a sterile mixture of sterile cefpirome sulphate and sodium carbonate.

Cefpirome Sulphate contains not less than 95.0 per cent and not more than 105.0 per cent of cefpirome sulphate, calculated on the dried and sodium carbonate free basis.

Category. Antibacterial.

Description. An off-white to pale yellow powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

B. It gives reaction (a) of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.0 to 8.0, determined in a 4.0 per cent w/v solution.

Absorbance. Not more than 0.3, determined on a 8.0 per cent w/v solution at 430 nm (2.4.7).

Heavy metals (2.3.13). 1 g complies with limit test for heavy metals, Method B (20 ppm).

Sodium carbonate. Disperse 0.29 g of the substance under examination in *water* and dilute to 250 ml with *water*. Dilute with *water* to get solution containing 12.5 µg per ml of sodium carbonate. To 10 ml of the solution add 10 ml of *potassium chloride solution* (1.907 per cent w/v), dilute to 100 ml with *water* and determine the absorbance at 589 nm by atomic absorption spectrophotometery (2.4.2, Method A) using *sodium solution AAS*, suitably diluted with *water* for the reference solution.

1 g of Na is equivalent to 2.305 g of Na_2CO_3.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Solvent mixture. 90 volumes of mobile phase A and 10 volumes of mobile phase B.

Test solution. Disperse 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *cefpirome sulphate IPRS* in the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (c). To 10 ml of reference solution (a), add 10 ml of the solvent mixture. Heat the solution at 75° on water-bath for 1 hour, cool.

Chromatographic system

- a stainless steel column 2.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 2.84 g of *disodium hydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.0 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent v/v)
0	99	1
5	94	6
10	94	6
20	80	20
25	80	20
30	70	30
37	70	30
40	40	60
50	40	60
60	99	1
70	99	1

Inject reference solution (c). The test is not valid unless the resolution between the principal peak and thermally degraded impurity peak is not less than 5.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary

peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent).

Loss on drying (2.4.19). Not more than 5.0 per cent determined on 1.0 g by drying under vaccum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of cefpirome.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *cefpirome sulphate IPRS* in the mobile phase.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c). To 10 ml of reference solution (a), add about 10 ml of the mobile phase. Heat the solution at 75° on water-bath for about an hour and cool.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of buffer solution prepared by dissolving 3.12 g of *sodium dihydrogen orthophosphate* in 100 ml of *water* and 10 volumes of *acetonitrile*, adjusted to pH 5.6 with 1 per cent w/v solution of *sodium hydroxide*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

The relative retention time with reference to cefpirome for cyclopentenpyridine is about 2.8 and for thermally degraded impurity is about 1.84.

Inject reference solution (b) and (c). The test is not valid unless the resolution between the principal peak and thermally degraded impurity peak is not less than 5.0 and the resolution between the thermally degraded impurity and cyclopentenpyridine is not less than 2.0 in the chromatogram obtained with reference solution (c). In the chromatogram obtained with reference solution (b), the relative standard deviation for replicate injections is not more than 1.0 per cent and the tailing factor is not more than 1.5.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{22}H_{22}N_6O_5S_2, H_2SO_4$.

Storage. Store protected from light and moisture.

Cefpirome Injection

Cefpirome Sulphate Injection

Cefpirome Injection is sterile material consisting of Cefpirome sulphate with or without auxiliary substances. It is filled in sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of Water for Injections immediately before use.

The constituted solution complies with the requirements for clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefpirome Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefpirome, $C_{22}H_{22}N_6O_5S_2$.

The contents of the sealed container comply with requirements stated under Parenteral Preparations (Powders for Injections) and with the following requirement.

Usual strengths. 250 mg; 500 mg; 1000 mg per vial.

Description. An off-white to pale yellow powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

B. It gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.0 to 8.0, determined in a 4.0 per cent w/v solution of cefpirome.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Solvent mixture. 90 volumes of mobile phase A and 10 volumes of mobile phase B.

Test solution. Disperse a quantity of powder containing 50 mg of Cefpirome Sulphate with 20 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *cefpirome sulphate IPRS* in the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (c). Transfer 10 ml of reference solution (a) to 50-ml volumetric flask, add about 10 ml of the solvent mixture. Heat the solution at 75° on water-bath for about an hour and cool.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 2.84 g of *disodium hydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.0 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the condition given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent v/v)
0	99	1
5	94	6
10	94	6
20	80	20
25	80	20
30	70	30
37	70	30
40	40	60
50	40	60
60	99	1
70	99	1

Inject reference solution (c). The test is not valid unless the resolution between the principal peak and thermally degraded impurity peak is not less than 5.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of cefpirome.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying under vaccum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 1.0 per cent w/v *sodium hydroxide solution*, pH 7.0.

Test solution. Mix the content of 10 containers containing 100 mg of Cefpirome Sulphate with 20 ml of the mobile phase with the aid of ultrasound and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *cefpirome sulphate IPRS* in the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of buffer solution prepared by dissolving 3.12 g of *sodium dihydrogen orthophosphate* in 100 ml of *water* and 10 volumes of *acetonitrile*, adjusted to pH 5.6 with 1.0 per cent w/v solution of *sodium hydroxide*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0.

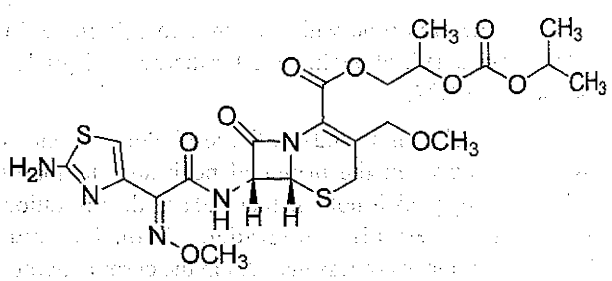
Inject reference solution (b) and the test solution.

Calculate the content of $C_{22}H_{22}N_6O_5S_2$ in the injection.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of Cefpirome.

Cefpodoxime Proxetil



$C_{21}H_{27}N_5O_9S_2$ Mol. Wt. 557.6

Cefpodoxime Proxetil is 1-(isopropoxycarbonyloxy)ethyl (6*R*, 7*R*)-7-[2-(2-amino-4-thiazolyl)-(*Z*)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate.

Cefpodoxime Proxetil contains not less than 690 µg and not more than 804 µg per mg of cefpodoxime, $C_{15}H_{17}N_5O_6S_2$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white to light brownish-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefpodoxime proxetil IPRS* or with the reference spectrum of cefpodoxime proxetil.

Tests

Specific optical rotation (2.4.22). +35° to +48°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. 20 volumes of *water* and 10 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 5 ml of *methanol* and dilute to 50.0 ml with the solvent mixture. This solution should be injected promptly.

Reference solution. Dissolve a quantity of *cefpodoxime proxetil IPRS* in the solvent mixture to obtain a solution containing about 10 µg per ml.

NOTE — A volume of methanol not exceeding 10 per cent of the total volume in the final solution may be used to facilitate dissolution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: A. *0.02 M ammonium acetate,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
10	68	32
40	68	32
80	50	50
85	50	50
90	25	75
95	25	75
100	90	10

Inject the reference solution. The retention time for the cefpodoxime proxetil *R*–epimer is between 37 and 42 minutes. The relative retention times for cefpodoxime proxetil *S*-epimer is about 0.9 and for cefpodoxime proxetil *R*-epimer is about 1.0, the resolution between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 4.0. The test is not valid unless the column efficiency for cefpodoxime proxetil *R*-epimer peak is not less than 15,000 theoretical plates.

Inject the test solution and measure the areas of all the peaks. Calculate the percentage of each impurity in the portion of cefpodoxime proxetil taken, from the expression, $100\ (r_i/r_s)$ where, r_i is the peak area for each impurity and r_s is the sum of the areas of all the peaks. Any peak at a relative retention time of about 0.86 is not more than 3.0 per cent, any peak at relative retention times of about 1.27, 1.39 is not more than 1.0 per cent, and other individual peaks having relative retention times higher than 2.0 is not more than 0.5 per cent and the sum of the areas of all the secondary peaks is not more than 6.0 per cent. Ignore any peak with an area less than 0.05 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Isomer ratio. Using the chromatogram of the test solution obtained in the Assay, calculate the ratio of the cefpodoxime proxetil *R*-epimer peak response to the sum of the peak responses of the cefpodoxime proxetil *S*-epimer peak and the cefpodoxime proxetil *R*-epimer peak: the ratio is between 0.5 and 0.6.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water* and 40 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 10 ml of *methanol*, dilute to 100.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture and filter.

Reference solution. Dissolve 25 mg of *cefpodoxime proxetil IPRS* in 5 ml of *methanol*, dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: A mixture of 60 volumes of *0.02 M ammonium acetate* and 40 volumes of *acetonitrile,*
- flow rate: 2 ml per minute,

- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative retention time for cefpodoxime proxetil *S*-epimer is about 0.9 and for cefpodoxime proxetil *R*-epimer is about 1.0. The resolution between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 2.5, the tailing factor for cefpodoxime proxetil *R*-epimer is not more than 1.5 and the relative standard deviation determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{17}N_5O_6S_2$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Cefpodoxime Oral Suspension

Cefpodoxime Proxetil Oral Suspension

Cefpodoxime Oral Suspension is a mixture consisting of Cefpodoxime Proxetil with buffering agents and other excipients. It contains a suitable flavouring agent.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Cefpodoxime Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefpodoxime, $C_{15}H_{17}N_5O_6S_2$.

Usual strengths. 50 mg per 5 ml; 100 mg per 5 ml.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cefpodoxime $C_{15}H_{17}N_5O_6S_2$.

Identification

In the Assay, the principal peaks of cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

pH (2.4.24). 4.0 to 5.5.

Water (2.3.43). Not more than 1.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water* and 40 volumes of *acetonitrile*.

Test solution. Transfer a weighed a quantity containing 50 mg of cefpodoxime, disperse in 10 ml of *water*, add 20 ml of *acetonitrile*, mix with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture and filter.

Reference solution. Dissolve a quantity of *cefpodoxime proxetil IPRS* in the solvent mixture to obtain a solution containing about 30 µg per ml.

NOTE — A volume of methanol not exceeding 10 per cent of the total volume in the final solution may be used to facilitate dissolution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: a mixture of 60 volumes of *0.02 M ammonium acetate* and 40 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative retention time for cefpodoxime proxetil *S*-epimer is about 0.9 and for cefpodoxime proxetil *R*-epimer is about 1.0. The resolution between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 2.5, the tailing factor for cefpodoxime proxetil *R*-epimer is not more than 1.5 and the relative standard deviation determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{15}H_{17}N_5O_6S_2$, weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states (1) the quantity of active ingredient in terms of the equivalent amount of cefpodoxime; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

Cefpodoxime Tablets

Cefpodoxime Proxetil Tablets

Cefpodoxime Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefpodoxime, $C_{15}H_{17}N_5O_6S_2$.

Usual strengths. 50 mg; 100 mg; 200 mg.

Identification

In the Assay, the principal peaks of cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a solution prepared by dissolving 3.03 g of *glycine* and 3.37 g of *sodium chloride* in about 500 ml of *water*, adding cautiously with swirling 0.8 ml of *hydrochloric acid*, adjusted to pH 3.0 and diluting to 1000 ml with *water*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 259 nm (2.4.7). Calculate the content of $C_{15}H_{17}N_5O_6S_2$ in the medium from the absorbance obtained from a solution of known concentration of *cefpodoxime proxetil IPRS* prepared by dissolving in minimum quantity of *methanol* and diluted with the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{15}H_{17}N_5O_6S_2$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water* and 40 volumes of *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 50 mg of cefpodoxime, disperse in 100.0 ml of the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture and filter.

Reference solution. Dissolve a quantity of *cefpodoxime proxetil IPRS* in the solvent mixture to obtain a solution containing about 30 µg per ml.

NOTE — A volume of methanol not exceeding 10 per cent of the total volume in the final solution may be used to facilitate dissolution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: a mixture of 60 volumes of *0.02 M ammonium acetate* and 40 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

The test is not valid unless the relative retention time for cefpodoxime proxetil *S*-epimer is about 0.9 and for cefpodoxime proxetil *R*-epimer is about 1.0.

Inject the reference solution. The test is not valid unless the resolution between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 2.5, the tailing factor for cefpodoxime proxetil *R*-epimer is not more than 1.5 and the relative standard deviation determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks for replicate injections is not more than 2.0 per cent.

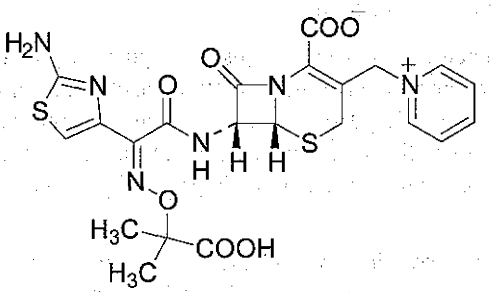
Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{17}N_5O_6S_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of Cefpodoxime. If the tablets are dispersible, the tablets should be dispersed in water immediately before use.

Ceftazidime



, $5H_2O$

$C_{22}H_{22}N_6O_7S_2,5H_2O$ Mol. Wt. 636.6

Ceftazidime is pentahydrate of the inner salt of (7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)-acetamido]-3-(1-pyridinomethyl)-3-cepham-4-carboxylate pentahydrate.

Ceftazidime contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{22}H_{22}N_6O_7S_2$, calculated on the dried basis.

Category. Antibacterial.

Description. A white to cream-coloured, crystalline powder.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 4.0, determined in a 0.5 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination to 20.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (a). A 0.00125 per cent w/v solution of *Δ-2-ceftazidime* (*ceftazidime impurity A IPRS*) in the mobile phase.

Reference solution (b). A 0.00125 per cent w/v solution of *ceftazidime IPRS* in reference solution (a).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 7 volumes of *acetonitrile* and 93 volumes of a 2.26 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 3.9 with *orthophosphoric acid*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 255 nm,
- injection volume: 20 µl.

Inject reference solution (b). Adjust the sensitivity of the system so that the heights of the 2 peaks in the chromatogram obtained are at least 50 per cent of the full scale of the recorder. The test is not valid unless in the resolution between the peaks due to ceftazidime and ceftazidime impurity A is not less than 5.9.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Pyridine. Not more than 500 ppm.

Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 0.5 g of the substance under examination in 100.0 ml of 10 per cent v/v solution of *phosphate buffer pH 7.0.*

Reference solution (a). Dissolve 1 g of *pyridine* in 100.0 ml of *water*. Dilute 5.0 ml of the solution to 200.0 ml with *water*. To 1.0 ml of the solution, add 10 ml of *phosphate buffer pH 7.0* and further dilute to 100 ml with *water*.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with 10 per cent v/v solution of *phosphate buffer pH 7.0*. To 1.0 ml of the solution, add 20 ml of reference solution (a) and further dilute to 200 ml with a 10 per cent v/v solution of *phosphate buffer pH 7.0.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 8 volumes of 2.88 per cent w/v solution of *ammonium dihydrogen orthophosphate* in *water*, previously adjusted to pH 7.0 with *ammonia*, 24 volumes of *acetonitrile* and 68 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 255 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to ceftazidime and pyridine is not less than 7.0.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Loss on drying (2.4.19). 13.0 to 15.0 per cent, determined on 0.3 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 30 mg of the substance under examination in 2.5 ml of *phosphate buffer pH 7.0*, dilute to 25.0 ml with *water* and mix. Protect the solution from light. Immediately before chromatography, dilute 5.0 ml of the solution to 50.0 ml with *water.*

Reference solution. Treat 30 mg *ceftazidime IPRS* in a similar manner.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 100 ml of *phosphate buffer pH 7.0* and 20 ml of *acetonitrile* diluted to 1000 ml with *water*,

- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{22}N_6O_7S_2$.

Ceftazidime intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.10 Endotoxin Unit per mg.

Ceftazidime intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture.

Ceftazidime for Injection

Ceftazidime for Injection is a sterile mixture of Sterile Ceftazidime and Sodium Carbonate or Arginine.

Ceftazidime for Injection contains not less than 90.0 per cent and not more than 105.0 per cent of ceftazidime, $C_{22}H_{22}N_6O_7S_2$, on the dried and sodium carbonate or arginine-free basis, and not less than 90.0 per cent and not more than 120.0 per cent of the labeled amount of ceftazidime, $C_{22}H_{22}N_6O_7S_2$.

Usual strengths. 500 mg; 1 g.

Description. A white or almost white powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts and reaction A of carbonates (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5, determined in a solution containing 100 mg of anhydrous ceftazidime per ml.

Pyridine. Not more than 0.4 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity containing about 0.5 g of ceftazidime and dissolve in sufficient *mixed phosphate buffer pH 7.0* to produce 100.0 ml.

Reference solution. Weigh accurately about 0.2 g of *pyridine* and dissolve in sufficient *water* to produce 100.0 ml. Immediately prior to chromatography add to 2.0 ml of the resulting solution sufficient *mixed phosphate buffer pH 7.0* to produce 200.0 ml and mix well.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 10 volumes of a 2.88 per cent w/v solution of *ammonium dihydrogen phosphate* previously adjusted to *pH 7.0* with *dilute ammonia solution*, 30 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation of replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of pyridine.

Content of arginine *(if present).*

Test solution. Dissolve accurately a quantity of Ceftazidime for Injection in *water* to obtain a solution containing 0.02 per cent w/v of Ceftazidime.

Reference solution. A solution containing 0.02 per cent w/v, each of, *ceftazidime pentahydrate IPRS* and *l-arginine IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with dihydroxypropane bonded to porous silica (3 to 10 µm), attached with a saturator pre-column 50 cm x 4.6 mm, packed with porous silica,
- mobile phase: a mixture of 25 volumes of a buffer solution prepared by dissolving 1.15 g of *monobasic ammonium phosphate* in 1000 ml of *water*, adjusted to pH 2.0 with *orthophosphoric acid*, and 75 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 206 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peak due to ceftazidime and arginine is not less than 6.0 and the tailing factor is not more than 4.0 for arginine peak.

Inject the reference solution and the test solution.

Calculate the content of arginine.

Sodium carbonate. Weigh accurately a quantity containing about 50 mg of anhydrous ceftazidime and dissolve in sufficient *water* to produce 100.0 ml. Dilute the resulting solution appropriately with *water* and determine by Method A for flame photometry (2.4.4), measuring at 589 nm or by Method A for atomic absorption spectrophotometry (2.4.2), using *sodium solution FP*, suitably diluted with *water* for the reference solutions.

1 g of Na is equivalent to 2.305 g of Na_2CO_3.

Bacterial endotoxins (2.2.3). Not more than 0.10 Endotoxin Unit per mg of ceftazidime.

Sterility (2.2.11). Complies with the test for sterility.

Loss on drying (2.4.19). Not more than 12.5 per cent (if contains arginine) and not more than 13.5 per cent (if contains sodium carbonate), determined on 0.3 g by drying in vacuum at a pressure not exceeding 5 mm of Hg at 25° for 4 hours. Where it contains sodium carbonate, heat the residue in vacuum at a pressure not exceeding 5 mm of Hg at 100° an additional 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents 10 containers. Dissolve a quantitity of the mixed contents containing 50 mg of anhydrous ceftazidime dissolve in water and dilute to 50.0 ml with the same solvent. Protect the solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with *water*.

Reference solution. Dissolve about 29 mg *ceftazidime IPRS* in 2.5 ml of *mixed phosphate buffer pH 7.0* and dilute to 25.0 ml with water. Protect the solution from light. Immediately before chromatography, dilute 5.0 ml to 50.0 ml with *water*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 100 ml of *phosphate buffer pH 7.0* and 20 ml of *acetonitrile* diluted to 1000 ml with *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

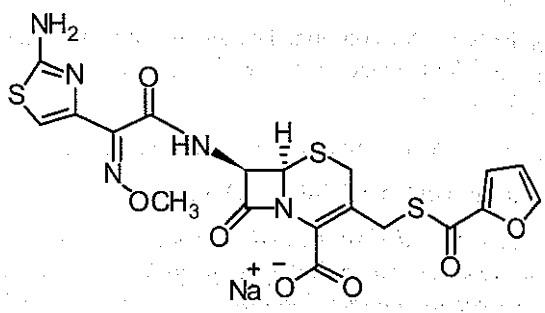
Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{22}N_6O_7S_2$.

Storage. Store in sterile containers, sealed so as to exclude microorganisms, protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of ceftazidime.

Ceftiofur Sodium



$C_{19}H_{16}N_5NaO_7S_3$ Mol. Wt. 545.5

$C_{19}H_{16}N_5NaO_7S_3,H_2O$ Mol. Wt. 563.5

$C_{19}H_{16}N_5NaO_7S_3,3H_2O$ Mol. Wt. 599.5

Ceftiofur Sodium is Sodium 7-[(*Z*)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]-3-(2-furoylthiomethyl)-3-cephem-4-carboxylate.

Ceftiofur Sodium contains not less than 82.0 per cent and not more than 102.0 per cent of $C_{19}H_{16}N_5NaO_7S_3$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. An off white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ceftiofur sodium IPRS* or with the reference spectrum of ceftiofur sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1).

pH (2.4.24). 5.0 to 7.5 determined in a 5.0 per cent w/v solution.

Specific optical rotation (2.4.22). – 70.0° to – 60.0°, determined in a 1.0 per cent w/v solution.

Absorbance. Absorbance of 2.0 per cent w/v solution at 420 nm (2.4.7) is not more than 0.3.

High molecular weight impurities. Not more than 12.0 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 15 ml of the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *ceftiofur sodium IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: dissolve 0.68 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.5 with *dilute potassium hydroxide solution*, add 10 g of *sodium lauryl sulphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The retention time of ceftiofur peak is about 3.0 minutes and high molecular weight impurities is about 1.7, 2.0 and 2.1.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of high molecular weight impurities by the area normalization using relative responses factor of 0.62.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 60 volumes of a 0.35 per cent w/v solution of *disodium hydrogen orthophosphate dihydrate*, adjusted to pH 8.0 with *orthophosphoric acid* and 40 volumes of *acetonitrile*.

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 100 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *ceftiofur sodium IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 95 volumes of a 0.1 per cent v/v solution of *trifluoroacetic acid* in *water* and 5 volumes of mobile phase B,
  B. a 0.1 per cent v/v solution of *trifluoroacetic acid* in *acetonitrile*,
- a gradient programme using the conditions given below;
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
15	82	18
50	82	18
110	40	60
111	100	0
120	100	0

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 5500 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than the 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent). Ignore any peaks with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (2.3.43). Not more than 2.0 per cent for anhydrous form, not more than 4.0 per cent for monohydrate form and not more than 10.0 per cent for trihydrate form, determined on 1.0 g, 0.5 g and 0.2 g.

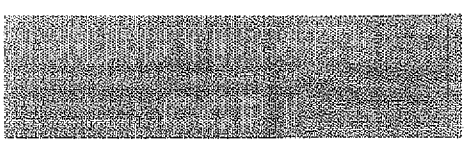
Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of a 0.35 per cent w/v solution of *disodium hydrogen orthophosphate dihydrate*, adjusted pH to 8.0 with *orthophosphoric acid* and 40 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solutuion of *ceftiofur sodium IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of a 0.1 per cent v/v solution of *trifluoroacetic acid* in *water* and 25 volumes a 0.1 per cent v/v solution of *trifluoroacetic acid* in *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume: 20 µl.

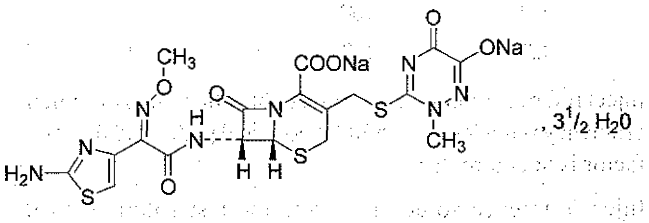


Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{16}N_5NaO_7S_3$.

Ceftriaxone Sodium



$C_{18}H_{16}N_8Na_2O_7S_3,3½H_2O$ Mol. Wt. 662.0

Ceftriaxone sodium is disodium (6*R*,7*R*)-7-[[(*Z*)-(2-aminothiazol-4-yl)(methoxyimino)acetyl]amino]-3-[[(2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl) sulphanyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hemiheptahydrate.

Ceftriaxone sodium contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{18}H_{16}N_8Na_2O_7S_3$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or yellowish, crystalline powder, slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ceftriaxone sodium IPRS* or with the reference spectrum of ceftriaxone sodium.

B. Gives reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. Dissolve 2.4 g in 20 ml of *carbon dioxide-free water* (Solution A). Dilute 2 ml of solution A to 20 ml with *water*; the resulting solution is clear (2.4.1) and not more intensely coloured than reference solution BYS5 or YS5 (2.4.1).

pH (2.4.24). 6.0 to 8.0, determined in solution A.

Specific optical rotation (2.4.22). –170.0° to –155.0°, determined in a 1.0 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30.0 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A 0.03 per cent w/v solution of *ceftriaxone sodium IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v, each of, *ceftriaxone sodium IPRS* and *ceftriaxone sodium E- isomer IPRS* in the mobile phase.

Reference solution (c). Dilute 1.0 ml of the reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 2.0 g of *tetradecylammonium bromide* and 2.0 g of *tetraheptylammonium bromide* in a mixture of 440 ml of *water*, 55 ml of *0.067 M mixed phosphate buffer solution pH 7.0*, 5.0 ml of a buffer solution prepared by dissolving 20.17 g of *citric acid* in 800 ml of *water*, adjusted to pH 5.0 with *strong sodium hydroxide solution* and diluting to 1000.0 ml with *water*, and 500 ml of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to ceftriaxone and ceftriaxone sodium *E*-isomer is at least 3.0.

Inject reference solution (b) and (c) and the test solution. Continue the chromatography for twice the retention time of the ceftriaxone peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent); the sum of the areas of all such peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4.0 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Water (2.3.43). 8.0 per cent to 11.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{16}N_8Na_2O_7S_3$.

Ceftriaxone sodium intended for use in the manufacture of parenteral preparations without a further appropriate

procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.20 Endotoxin Unit per mg of ceftriaxone sodium.

Ceftriaxone Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with following requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture.

Labelling. The label states, where applicable, that the substance is free from bacterial endotoxins.

Ceftriaxone Injection

Ceftriaxone Injection is a sterile material consisting of Ceftriaxone Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Usual strengths. The equivalent of 250 mg; 500 mg; 1 g and 2 g of ceftriaxone.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ceftriaxone Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of ceftriaxone, $C_{18}H_{18}N_8O_7S_3$.

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ceftriaxone sodium IPRS* or with the reference spectrum of ceftriaxone sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

C. It gives the reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. A 1.2 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than reference solution BYS5 or YS5 (2.4.1).

pH (2.4.24). 6.0 to 8.0, determined in a 10.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of injection containing 30 mg of ceftriaxone in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A 0.03 per cent w/v solution of *ceftriaxone sodium IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v, each of, *ceftriaxone sodium IPRS* and *ceftriaxone sodium E-isomer IPRS* in the mobile phase.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

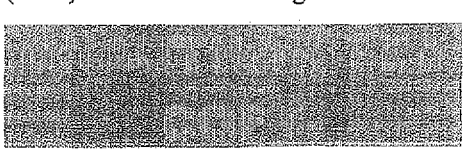
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Lichrosphere RP-18),
- mobile phase: dissolve 2 g of *tetradecylammonium bromide* and 2 g of *tetraheptylammonium bromide* in a mixture of 440 ml of *water*, 55 ml of *0.067 M mixed phosphate buffer pH 7.0*, 5 ml of a buffer prepared by dissolving 20.17 g of *citric acid* in 800 ml of *water*, adjusted to pH 5.0 with *10 M sodium hydroxide* and diluting to 1000 ml with *water*, and 500 ml of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 3.0.

Inject reference solution (c) and the test solution. Run the chromatogram at least twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the sum of the areas of all the secondary peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of ceftriaxone.

Water (2.3.43). Not more than 11.0 per cent, determined on 0.1 g.



Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{18}N_8O_7S_3$ in the injection.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label on the sealed container states the quantity of Ceftriaxone Sodium contained in it in terms of the equivalent amount of ceftriaxone.

Ceftriaxone and Sulbactam for Injection

Ceftriaxone Sodium and Sulbactam Sodium for Injection

Ceftriaxone and Sulbactam for Injection is a sterile material consisting of Ceftriaxone Sodium and Sulbactam Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ceftriaxone and Sulbactam for Injection contain ceftriaxone sodium and sulbactam sodium equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of ceftriaxone, $C_{18}H_{18}N_8O_7S_3$ and sulbactam, $C_8H_{11}NO_5S$.

Description. A white to off white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strengths. Ceftriaxone, 2000 mg and Sulbactam, 1000 mg; Ceftriaxone, 1000 mg and Sulbactam, 500 mg; Ceftriaxone, 500 mg and Sulbactam, 250 mg; Ceftriaxone, 250 mg and Sulbactam, 125 mg; Ceftriaxone, 125 mg and Sulbactam, 62.5 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

Tests

pH (2.4.24). 4.5 to 8.0, determined in 10 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the injection containing 30 mg of Ceftriaxone in 60 ml of *water* and dilute to 100.0 ml with *water*.

Reference solution. A 0.03 per cent w/v solution of *ceftriaxone sodium IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3V),
- sample temperature: 10°,
- mobile phase: A. a mixture of 95 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 5 volumes of *methanol*,

  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
5	95	5
15	80	20
25	80	20
30	60	40
50	60	40
55	95	5
60	95	5

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 1.0 per cent and the sum of the areas of all secondary peaks is not more than 5.0 per cent, calculated by area normalisation.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of ceftriaxone.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 10 vials. Disperse a quantity of the mixed contents containing 250 mg of Ceftriaxone in

100.0 ml of *water*. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (a). Weigh a suitable quantity of *ceftriaxone sodium IPRS* and dissolve in *water* to obtain a solution containing 0.025 w/v solution of ceftriaxone.

Reference solution (b). Weigh a suitable quantity of *sulbactam sodium IPRS* and dissolve in *water* to obtain a solution containing 0.025 w/v solution of sulbactam.

Reference solution (c). Dilute a suitable volume of reference solution (a) and reference solution (b) with the mobile phase to obtain a solution having the similar concentration to the test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, add 2 ml of *triethylamine* and mix, adjusted to pH 4.5 with *orthophosphoric acid* and 25 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

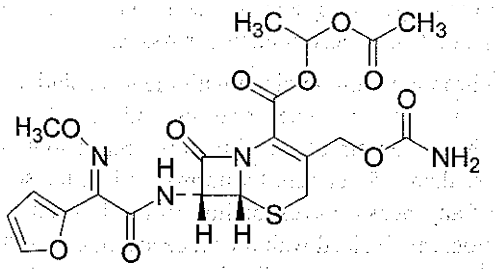
Inject reference solution (c). The test is not valid unless the tailing factor is not more than 2.0 for ceftriaxone peak and the relative standard deviation for replicate injections is not more than 2.0 for both the peaks.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{18}H_{18}N_8O_7S_3$ and $C_8H_{11}NO_5S$ in the injection.

Labelling. The label states the strength in terms of equivalent amount of ceftriaxone and sulbactam.

Cefuroxime Axetil



$C_{20}H_{22}N_4O_{10}S$ Mol. Wt. 510.5

Cefuroxime Axetil is a mixture of the 2 diastereoisomers of (1*RS*)-1-(acetyloxy)ethyl (6*R*,7*R*)-3-[(carbamoyloxy)methyl]-7-[[(*Z*)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

Cefuroxime Axetil contains not less than 79.8 per cent and not more than 84.8 per cent of cefuroxime, $C_{16}H_{16}N_4O_8S$, calculated on the anhydrous and acetone-free basis.

Category. Antibacterial.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefuroxime axetil IPRS* or with the reference spectrum of cefuroxime axetil.

B. In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks due to diastereomer A and B in the chromatogram obtained with reference solution (d).

Tests

Diastereoisomer ratio. Determine by liquid chromatography (2.4.14).

Use chromatographic system, test solution, reference solution (a), (b), (c) and (d), as described under Assay.

In the chromatogram obtained with the test solution, the ratio of the peak due to cefuroxime axetil diastereoisomer A to the sum of the peaks due to cefuroxime axetil diastereoisomers A and B is between 0.48 and 0.55 by the normalisation procedure.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Heat 5 ml of the test solution at 60° for one hour to generate the D^3-isomers.

Reference solution (c). Expose 5 ml of the test solution to ultraviolet light at 254 nm for 24 hours to generate *E*-isomers.

Reference solution (d). A 0.02 per cent w/v solution of *cefuroxime axetil IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with trimethylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 38 volumes of *methanol* and 62 volumes of a 2.3 per cent w/v solution of *ammonium dihydrogen phosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume: 20 µl.

The relative retention times with respect to cefuroxime axetil diastereoisomer A for cefuroxime axetil diastereoisomer B is about 0.9, for cefuroxime axetil D^3-isomers is about 1.2 and for *E*-isomers is about 1.7 and 2.1.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to cefuroxime axetil diastereoisomer A and cefuroxime axetil D^3-isomer is not less than 1.5.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to cefuroxime axetil D^3-isomers is not more than 1.5 times the sum of the area of the principal peaks in the chromatogram obtained with reference solution (a) (1.5 per cent), the area of peak corresponding to cefuroxime axetil E-isomers is not more than the sum of the area of the principal peaks in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of any other secondary peak is not more than 0.5 times the sum of the area of the principal peaks in the chromatogram obtained with reference solution (a) (0.5 per cent) and sum of all the secondary peaks is not more than 3 times the sum of the area of the principal peaks in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak with an area less than 0.05 times the sum of the area of the principal peaks in the chromatogram obtained with reference solution (a) (0.05 per cent).

Acetone (5.4). Not more than 1.1 per cent.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.4 g.

Assay. Determine by liquid chromatography (2.4.14).

Use chromatographic system, test solution and reference solution (d), as described under Related substances.

Inject reference solution (d). The test is not valid unless the resolution between the peaks corresponding to cefuroxime axetil diastereoisomers A and B is not less than 1.5 and the relative standard deviation for replicate injections for the sum of diastereomer A and B peaks is not more than 2.0 per cent.

Inject reference solution (d) and the test solution.

Calculate the content of $C_{16}H_{16}N_4O_8S$ as the sum of areas of the two diastereoisomer peaks.

1 mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

Storage. Store protected from light and moisture.

Cefuroxime Axetil Tablets

Cefuroxime Axetil Tablets contain Cefuroxime Axetil. They may be coated.

Cefuroxime Axetil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cefuroxime, $C_{16}H_{16}N_4O_8S$.

Usual strengths. 125 mg; 250 mg; 500 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.1 g of cefuroxime with 5 ml of *dichloromethane*, filter and evaporate the filtrate to dryness.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cefuroxime axetil IPRS* or with the reference spectrum of cefuroxime axetil.

B. In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks due to diastereomer A and B in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly. Use the filtrate, dilute if necessary, with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{16}H_{16}N_4O_8S$ in the medium from the absorbance obtained from a solution of known concentration of *cefuroxime axetil IPRS*.

Q. Not less than 70 per cent of the stated amount of $C_{16}H_{16}N_4O_8S$.

Related substances. Determine by liquid chromatography (2.4.14).

Use chromatographic system, test solution, reference solution (a), (b), and (c), as described under Assay.

In the chromatogram obtained with the test solution the sum of the areas of the pair of peaks corresponding to the *E*-isomers in the chromatogram obtained with reference solution (b) is not more than 1.5 per cent by normalisation, the sum of the areas of any peaks corresponding to the D^3-isomers in the chromatogram obtained with reference solution (a) is not more than 2.0 per cent by normalisation and the area of any other secondary peak is not more than 1.0 per cent by normalisation.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE – Prepare the solutions immediately before use.

Test solution. Disperse 10 tablets in *0.2 M ammonium dihydrogen orthophosphate* with the pH previously adjusted to 2.4 with *orthophosphoric acid*, using 10 ml per g of the stated content of cefuroxime. Immediately add sufficient *methanol* to produce a solution containing the equivalent of 0.5 per cent w/v of cefuroxime and shake vigorously. Filter and dilute a quantity of the filtrate with sufficient of the mobile phase to produce a solution containing 0.025 per cent w/v of cefuroxime.

Reference solution (a). Warm a quantity of the test solution at 60° for one hour or until sufficient impurities (D^3-isomers) have been generated.

Reference solution (b). Expose a quantity of the test solution to ultraviolet light at 254 nm for 24 hours or until sufficient impurities (*E*-isomers) have been generated.

Reference solution (c). A 0.03 per cent w/v solution of *cefuroxime axetil IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with particles of silica (5 µm) the surface of which has been modified by chemically-bonded trimethylsilyl groups (Such as Hypersil SAS),
- mobile phase: a mixture of 38 volumes of *methanol* and 62 volumes of *0.2 M ammonium dihydrogen orthophosphate*, adjusted, if necessary, so that the resolution between the peaks corresponding to the cefuroxime axetil diastereoisomers A and B in reference solution (c) and between the peaks corresponding to cefuroxime axetil diastereoisomer A and the cefuroxime axetil D^3-isomer in reference solution (a) is in each case not less than 1.5,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution, reference solution (a), (b) and (c). The retention time relative to cefuroxime axetil diastereoisomer A are approximately 0.9 for cefuroxime axetil diastereoisomer B, 1.2 for the cefuroxime axetil D^3-isomers and 1.7 and 2.1 for the *E*-isomers.

Calculate the content of $C_{16}H_{16}N_4O_8S$ as the sum of the areas of the two peaks corresponding to diastereoisomers A and B.

1 mg of $C_{20}H_{22}N_4O_{10}S$ is equivalent to 0.8313 mg of $C_{16}H_{16}N_4O_8S$.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of cefuroxime.

Cefuroxime Axetil and Potassium Clavulanate Tablets

Cefuroxime Axetil and Potassium Clavulanate Tablets contain Cefuroxime Axetil and Potassium Clavulanate or Potassium Clavulante Diluted. The tablets are coated.

Cefuroxime Axetil and Potassium Clavulanate Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amounts of cefuroxime, $C_{16}H_{16}N_4O_8S$ and clavulanic acid $C_8H_9NO_5$.

Usual strength. Cefuroxime, 250 mg and Cavulanic acid, 125 mg.

Identification

In the Assay, the retention time of the principal peaks in the chromatogram obtained with the test solution correspond to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus. No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the mobile phase.

Reference solution. Dissolve 34 mg of *cefuroxime axetil IPRS* and 14 mg of *lithium clavulanate IPRS* in 10 ml of *methanol* and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml to 50.0 ml with mobile phase.

Use the chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{16}N_4O_8S$ and $C_8H_8KNO_5$.

Q. Not less than 75 per cent of the stated amounts of $C_{20}H_{22}N_4O_{10}S$ and $C_8H_9NO_5$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of cefuroxime in 10 ml of *methanol* and dilute to 50.0 ml with mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve 34 mg of *cefuroxime axetil IPRS* and 14 mg of *lithium clavulanate IPRS* in 10 ml of *methanol*

and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml to 50.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 15°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 23.0 g of *ammonium di hydrogen phosphate* in 1000 ml *water* and 45 volumes of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent and the resolution between cefuroxime axetil and potassium clavulanate peaks is not less than 1.5

Inject the reference solution and the test solution.

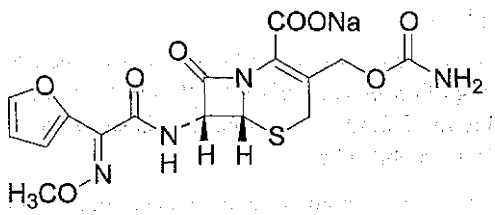
Calculate the contents of $C_{16}H_{16}N_4O_8S$ and $C_8H_9NO_5$ in the tablets.

1 mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labellilng. The label states the strength in terms of the equivalent amount of cefuroxime and clavulanic acid.

Cefuroxime Sodium



$C_{16}H_{15}N_4NaO_8S$ Mol. Wt. 446.4

Cefuroxime Sodium is sodium (7*R*)-3-carbamoyloxymethyl-7-[(*Z*)-furan-2-yl-2-methoxyiminoacetamido]-3-cephem-4-carboxylate.

Cefuroxime Sodium contains not less than 90.0 per cent and not more than 105.0 per cent of cefuroxime sodium, $C_{16}H_{15}N_4NaO_8S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or faintly yellow powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 6.0 to 8.5, determined in a 10.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Prepare the solutions immediately before use or keep at 2° to 8°.

Test solution. Dissolve 25 mg of the substance under examination in *water* and dilute to 25.0 ml with *water.*

Reference solution (a). Dissolve 25 mg of *cefuroxime sodium IPRS* in *water* and dilute to 25.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *water*. Heat 20 ml of the solution in a water–bath at 80° for 15 minutes. Cool and inject immediately.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *water.*

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with hexylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1 volume of *acetonitrile* and 99 volumes of an *acetate buffer pH 3.4*, prepared by dissolving 6.01 g of *glacial acetic acid* and 0.68 g of *sodium acetate* in *water* and diluting to 1000 ml with *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 273 nm,
- injection volume: 20 µl.

Inject reference solution (a).The test is not valid unless the resolution between the peaks due to cefuroxime and discarbamoyl cefuroxime (cefuroxime impurity A) is not less than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to cefuroxime impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (2.3.43). Not more than 3.5 per cent, determined on 0.15 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the *water* to produce 25.0 ml. Immediately transfer 5.0 ml of the solution to a 100-ml volumetric flask, add 20.0 ml of a 0.15 per cent w/v solution of *orcinol* (internal standard) in *water*, dilute to volume with *water* and mix.

Reference solution. Treat a quantity of *cefuroxime sodium IPRS* equivalent to 25 mg of cefuroxime in a similar manner.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with hexylsilane chemically bonded to totally porous silica particles (5 µm),
- mobile phase: a mixture of 100 volumes of *acetate buffer pH 3.4* and 10 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{15}N_4NaO_8S$.

Cefuroxime Sodium intended for use in the manufacture of parenteral preparations complies with the following additional requirements.

Bacterial endotoxins (2.2.3). Not more than 0.1 Endotoxin Unit per mg of cefuroxime sodium.

Sterility (2.2.11). Complies with the test for sterility, using the membrane filtration method.

Storage. Store protected from moisture. If it is intended for use in the manufacture of parenteral preparations, it should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of injectable preparations.

Cefuroxime Injection

Cefuroxime Sodium Injection

Cefuroxime Injection is a sterile material consisting of Cefuroxime Sodium, with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of a sealed container in the requisite amount of Water for Injections immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cefuroxime Injection contains a quantity of Cefuroxime Sodium equivalent to not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of cefuroxime, $C_{16}H_{16}N_4O_8S$.

Usual strengths: The equivalent of 250 mg, 750 mg and 1.5 g of cefuroxime.

Description. A white or faintly yellow powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with the reference solution.

B. It gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 6.0 to 8.5, determined in a 10.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 10 containers. Dissolve a quantity of the mixed content containing 0.1 g of cefuroxime to 100 ml of *water*.

Reference solution (a). A 0.1 per cent w/v solution of *cefuroxime sodium IPRS* in *water*.

Reference solution (b). Heat 20.0 ml of reference solution (a) in water bath at 60° for 10 minutes, cool.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm packed with silica chemically bonded to hexylsilane groups (5 µm) (Such as Spherisorb S5 C6),
- mobile phase: a mixture of 1 volume of *acetonitrile* and 99 volumes of *acetate buffer pH 3.4*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 273 nm,
- injection volume: 20 µl.

Inject reference solution (b). The chromatogram obtained shows peaks corresponding to cefuroxime and descarbamoyl-

cefuroxime. The test is not valid unless the resolution between the two principal peaks is not less than 2.0.

Inject reference solution (c) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak due to descarbamoyl-cefuroxime is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.1 Endotoxin Unit per mg of cefuroxime.

Water (2.3.43). Not more than 3.5 per cent, determined on 0.15 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 10 containers. Dissolve a quantity of the mixed contents containing 25 mg of cefuroxime and dissolve in sufficient *water* to produce 25.0 ml. Immediately transfer 5.0 ml of the resulting solution to a 100-ml volumetric flask, add 20.0 ml of a 0.15 per cent w/v solution of *orcinol* (internal standard) in *water,* dilute to volume with *water* and mix.

Reference solution. Treat a quantity of *cefuroxime sodium IPRS* equivalent to 25 mg of cefuroxime in a similar manner.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with hexylsilane chemically bonded to totally porous silica particles (5 µm),
- mobile phase: a mixture of 91 volumes of *acetate buffer pH 3.4* and 9 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

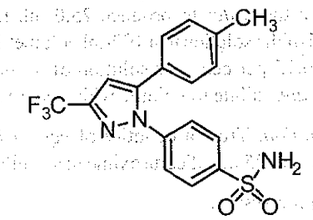
Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{16}N_4O_8S$ in the injection.

Storage. Store in tightly-closed containers protected from moisture at a temperature not exceeding 30°.

Labelling. The label on the sealed container states the quantity of Cefuroxime Sodium contained in it in terms of the equivalent amount of cefuroxime.

Celecoxib



$C_{17}H_{14}F_3N_3O_2S$ Mol. Wt. 381.4

Celecoxib is 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzene sulphonamide.

Celecoxib contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{14}F_3N_3O_2S$, calculated on the anhydrous basis.

Category. Cyclo-oxygenase inhibitor, analgesic, antiinflamatory.

Description. A white or almost white, crystalline or amorphous powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *celecoxib IPRS* or with reference spectrum of celecoxib.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *water* and 75 volumes of *methanol*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *celecoxib IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.006 per cent w/v, each of, *celecoxib impurity A IPRS* and *celecoxib impurity B IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 25.0 ml with reference solution (a).

Reference solution (c). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with phenylsilane bonded to porous silica (5 µm),

- column temperature: 60°
- mobile phase: a mixture of 10 volumes of *acetonitrile*, 30 volumes of *methanol* and 60 volumes of buffer solution prepared by dissolving 2.7g *potassium dihydrogen phosphate* in 1000 ml of *water,* adjusted to pH 3.0 with *orthophosphoric acid,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 25 µl.

Name	Relative retention time
Celecoxib impurity A[1]	0.9
Celecoxib (Retention time:about 27 minutes)	1.0
Celecoxib impurity B[2]	1.1

[1]4-[5-(3-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] benzenesulphonamide,

[2]4-[3-(4-methylphenyl)-5-(trifluoromethyl)-1*H*-pyrazol-1-yl] benzenesulphonamide.

Inject reference solution (b). The test is not valid unless resolution between the peaks due to celecoxib impurity A and celecoxib is not less than 1.8 and the resolution between the peaks due to celecoxib impurity B and celecoxib is not less than 1.8.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to celecoxib impurity A is not more than 4 times the area of principal peak in the chromatogram obtained with reference solution (c) (0.4 per cent), the area of any other secondary peak is not more than the area of principal peak in the chromatogram obtained with reference solution (c) (0.1per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area the area of principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.4 g.

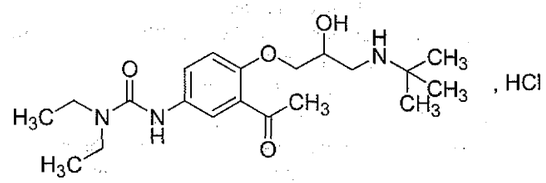
Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{14}F_3N_3O_2S$.

Storage. Store protected from moisture.

Celiprolol Hydrochloride



$C_{20}H_{33}N_3O_4.HCl$ Mol Wt. 416.0

Celiprolol Hydrochloride is (*RS*)-3-{3-Acetyl-4-[3-(*tert*.butylamino)-2-hydroxypropoxy]phenyl}-1,1-diethylurea hydrochloride.

Celiprolol Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{20}H_{33}N_3O_4.HCl$ calculated on the dried basis.

Category. Antihypertensive.

Description. A white to very slightly yellow crystalline powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *celiprolol hydrochloride IPRS* or with the reference spectrum of celiprolol hydrochloride.

B. It gives reaction (a) of chlorides (2.3.1).

Tests

Optical rotation (2.4.22). – 0.1° to + 0.1°, determined on 10.0 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 100 mg of the substance under examination in 20.0 ml of mobile phase A.

Reference solution (a). A solution containing 0.004 per cent w/v, each of, the substance under examination and *acebutolol hydrochloride IPRS* in mobile phase A.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 91 ml of *tetrahydrofuran*, 63 ml of *acetonitrile*, 0.6 ml of *pentafluoropropanoic*

acid and 0.2 ml of *trifluoroacetic acid*, dilute to 1000 ml with *water*,

B. *acetonitrile*,

- a gradient programme using the conditions given below,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 232 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
50	80	20
51	80	20
65	100	0

Name	Relative retention time	Correction factor
Celiprolol impurity A[1]	0.3	4.0
Celiprolol impurity D[2]	0.7	—
Celiprolol (Retention time: about 10 minutes)	1.0	—
Celiprolol impurity G[3]	1.2	—
Celiprolol impurity B[4]	1.4	1.5
Celiprolol impurity F[5]	1.6	0.5
Celiprolol impurity C[6]	2.2	—
Celiprolol impurity H[7]	2.5	—
Celiprolol impurity I[8]	2.5	1.7
Celiprolol impurity E[9]	3.9	2.3

[1]1-[5-amino-2-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]phenyl] ethanone,

[2]3-[3-acetyl-4-[(2*RS*)-3-(diethylamino)-2- hydroxypropoxy] phenyl]-1,1-diethylurea,

[3]3-[3-acetyl-4-[[(*RS*)-oxiranyl]methoxy]phenyl]-1,1-diethylurea,

[4]1,3-bis[3-acetyl-4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy] phenyl]urea,

[5]3-(3-acetyl-4-hydroxyphenyl)-1,1-diethylurea,

[6]1-[3-acetyl-4-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxy-propoxy]phenyl]-3-(1,1-dimethylethyl) urea,

[7]3-[3- acetyl-4-[(2*RS*)-3-bromo-2- hydroxypropoxy]phenyl]-1,1-diethylurea,

[8]1-acetyl-1-(4-ethoxyphenyl)-3,3-diethylurea,

[9]1,1'-[[(1,1-dimethylethyl)imino]bis[(2-hydroxypropane-1,3-diyl)oxy(3-acetyl-1,4- phenylene)]]bis(3,3-diethylurea).

Inject reference solution (a). The test is not valid unless the resolution between the peak corresponding to celiprolol and acebutolol is not less than 4.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the area of not more than 1 such peak is more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 5 times area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hour.

Assay. Dissolve 0.35 g in 50.0 ml of *ethanol (95 per cent)* under nitrogen atmosphere, add 1 ml of *0.1 M hydrochloric acid* and titrate with *0.1 M sodium hydroxide* determining the end point potentiometrically (2.4.25). Read the volume added between the two points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.04160 g of $C_{20}H_{33}N_3O_4HCl$.

Storage. Store protected from light.

Celiprolol Tablets

Celiprolol Hydrochloride Tablets

Celiprolol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of celiprolol hydrochloride, $C_{20}H_{33}N_3O_4,HCl$.

Usual strengths. 100 mg; 200 mg; 400 mg.

Identification

Mix with the aid of ultrasound a quantity of the powdered tablets containing 200 mg of Celiprolol Hydrochloride with 100 ml of *dichloromethane* for 30 minutes, filter, remove the dichloromethane using a rotary evaporator and dry the residue over *phosphorus pentoxide* at 110° at a pressure not exceeding 2 kPa for 1 hour. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *celiprolol hydrochloride IPRS* or with the reference spectrum of celiprolol hydrochloride.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, suitably with the dissolution medium and measure the absorbance at the maximum at about 231 nm (2.4.7).

Calculate the content of $C_{20}H_{33}N_3O_4$,HCl in the medium from the absorbance obtained from a solution of known concentration of *celiprolol hydrochloride IPRS*.

Q. Not less than 75 per cent of the stated amount $C_{20}H_{33}N_3O_4$,HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.1 g of Celiprolol Hydrochloride in 100.0 ml of the mobile phase with the aid of ultrasound for 15 minute, cool and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.1 per cent w/v of *celiprolol hydrochloride IPRS* in *water*, 5 drops of *5 M sodium hydroxide* and heat at 70° for 20 minutes (generates celiprolol impurity A).

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of 0.025 *M sodium dihydrogen phosphate monohydrate* adjusted to pH 3.0 with *3 M orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at: 233 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the peak due to celiprolol impurity A (3-acetyl-4-3-(1,1-dimethyl-ethylamino)-2-hydroxypropoxybutyranilide) is resolved from the solvent front.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to celiprolol impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of not more than one such peak has an area not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than a fifth of the area of the peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of Celiprolol Hydrochloride in 100 ml of the mobile phase with the aid of ultrasound for 15 minutes, cool and filter. Dilute 1.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution. A 0.002 per cent w/v solution of *celiprolol hydrochloride IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{33}N_3O_4$,HCl in the tablets.

Storage. Store protected from light.

Cellulose Acetate Phthalate

Cellacephate; Cellacefate

Cellulose Acetate Phthalate is a cellulose, some of the hydroxyl groups of which are esterified by acetyl groups and others by hydrogen phthaloyl groups.

Cellulose Acetate Phthalate contains not less than 17.0 per cent and not more than 26.0 per cent of acetyl groups, C_2H_3O and not less than 30.0 per cent and not more than 40.0 per cent of hydrogen phthaloyl groups, $C_8H_5O_3$ both calculated on the anhydrous, acid free basis.

Category. Pharmaceutical aid (for enteric coating of tablets).

Description. A white, free-flowing powder or colourless flakes; odourless or with a faint odour of acetic acid; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cellulose acetate phthalate IPRS* or with the reference spectrum of cellulose acetate phthalate.

B. To about 10 mg contained in a small test-tube add 10 mg of *resorcinol*, mix, add 0.5 ml of *sulphuric acid* and heat in a liquid paraffin bath at 160° for 3 minutes. Cool and pour the solution into a mixture of 25 ml of *sodium hydroxide solution* and 200 ml of *water*; the solution shows a vivid green fluorescence.

Tests

Viscosity (2.4.28). 50 mm^2s^{-1} to 90 mm^2s^{-1}, determined in the following manner. Weigh accurately about 15 g, previously dried at 105° for 2 hours, and dissolve in 85 g of a mixture of 249 parts of *dry acetone* and 1 part of *water*. Determine at 25° the viscosity of the resulting solution by Method A, using a size D viscometer.

Appearance of a film. Dissolve 3.0 g in 17 ml of *acetone* with a water content of 0.35 to 0.45 per cent w/w. Allow 1 ml of the solution to flow over a glass plate and dry; a thin, colourless, transparent and glossy film is produced.

Free acid. Not more than 3.0 per cent, calculated as phthalic acid, $C_8H_6O_4$, on the anhydrous basis and determined in the following manner. Weigh accurately 1.0 g, in fine powder, shake for 5 minutes with 100 ml of boiled *water* and filter. Wash the flask and the filter with two quantities, each of 10 ml, of *water*. Combine the filtrate and washings, add 5 drops of *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* until a faint pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0083 g of phthalic acid.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 5.0 per cent, using 0.5 g dissolved in 20 ml of a mixture of equal volumes of *anhydrous methanol* and *chloroform*.

Assay. *For acetyl groups* — Weigh accurately about 0.1 g and heat on a water-bath for 30 minutes with 25.0 ml of *0.1 M sodium hydroxide* under reflux. Cool, add 5 drops of *phenolphthalein solution* and titrate with *0.1 M hydrochloric acid* until the colour is discharged. Carry out a blank titration. Calculate the acetyl groups, C_2H_3O, from the expression

$$0.43c/w-(0.578p+0.518s)$$

where, c = volume, in ml, of *0.1 M hydrochloric acid* consumed

w = weight, in g, of the sample, calculated with reference to anhydrous substance,

p = percentage of hydrogen phthaloyl groups as determined in Assay for hydrogen phthaloyl groups,

s = percentage of free acid.

For hydrogen phthaloyl groups — Weigh accurately about 0.4 g (calculated on the anhydrous basis) and dissolve without heating in 20 ml of *2-methoxyethanol*, previously neutralised in the presence of 5 drops of *phenolphthalein solution*. Titrate with *0.1 M sodium hydroxide* until a faint pink colour is produced. Calculate the hydrogen phthaloyl groups, $C_8H_5O_3$, from the expression

$$1.49b/w-1.795s$$

where, b = volume, in ml, of *0.1 M sodium hydroxide* consumed,

w = weight, in g, of the sample, calculated with reference to the anhydrous substance,

s = percentage of free acid.

Storage. Store protected from moisture at a temperature between 8° and 15°.

Hard Cellulose Capsule Shells

Hard Cellulose Capsule Shells are soluble containers for incorporation of drugs and/or medicaments, usually in the form of powders, pellets or granules, semisolids or liquids, and are commonly intended for oral administration. The shells are acted upon by digestive fluids and the filled contents are released. The shells are composed of Hydroxypropyl-methylcellulose or any other cellulose derivatives and water.

The capsule shell may contain gelling agents, gelling aids and other additives such as plasticizers, humectants, surfactants, dispersing agents, gliding agents, lubricating agents, flavouring agents, antimicrobial agents, sweetening agents, opacifying agents and one or more colouring agents permitted under the Drugs and Cosmetics Rules,1945.

Category. Pharmaceutical aid.

Description. Hard Cellulose Capsule Shells consist of two cylindrical, telescoping pieces (cap and body), one end of which is rounded and closed, and the other end is open. Shapes other than cylindrical can also be formed as per the requirements. The two pieces are coloured or uncoloured, of identical or different colours, transparent, translucent or opaque, and printed or unprinted or bear other surface markings. The cap overlaps the body and maintains a tight friction closure. The closure may be strengthened by suitable means.

The shells are of various sizes, usually designated by different numbers, 5 being the smallest and 000 the biggest. Shells of special lengths, shapes and designations are also available. The shells are smooth and uniform in size, shape and colour. Guidelines on dimensions in respect of different sizes of commonly used capsules are given in the table (5.8.2).

Identification

A. Add capsules, quantity equivalent to 1.0 g under constant stirring, into 50 ml of *carbon dioxide-free water* previously heated to 90°. Allow to cool, dilute to 100 ml with *carbon dioxide-free water* and continue stirring until solution is complete (solution A). If the capsules are not transparent, centrifuge the solution A & consider only supernatant liquid as solution A for further tests. Heat 10 ml of solution A in a water-bath with stirring. At temperatures above 50°, the solution becomes cloudy or a flocculent precipitate is formed. On cooling, the solution becomes clear or slightly opalescent.

B. To 10 ml of solution A add 10 ml of *1 M sodium hydroxide* or *1 M hydrochloric acid*; in either case the mixture remains stable.

C. To 10 ml of solution A add 0.3 ml of *2 M acetic acid* and 2.5 ml of a 10 per cent w/v solution of *tannic acid*; a yellowish white, flocculent precipitate is produced which dissolves in *6 M ammonia*.

D. Place 1 ml of solution A on a glass plate. After evaporation of the *water* a thin film is produced.

E. Without heating add 20 ml of solution A in 15 ml of a 70 per cent w/w solution of *sulphuric acid*, pour the solution with stirring into 80 ml of iced *water*. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of *sulphuric acid*, added drop wise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of *ninhydrin* in 100 ml of a 4.55 per cent w/v solution of *sodium metabisulphite*, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

F. Boil one capsule shell with 20 ml of *water*, allow to cool and centrifuge. To 5 ml of the supernatant liquid add 1 ml of *picric acid solution*; no precipitate is produced. Distinction from gelatin.

Tests

Odour. Keep 100 capsule shells in a well-closed bottle for 24 hours at a temperature between 30° and 40°; the shells do not develop any foreign odour.

NOTE — In order to ensure that the quality of the shells is not affected by temperature and humidity, the capsule shells should be conditioned at a temperature of 25° ± 2° and a relative humidity of 50 ± 5 per cent for not less than 12 hours before conducting the test for Average weight.

Average weight. Weigh 100 capsule shells and determine the average weight of a capsule. The average weight is within ± 10 per cent of the target weight shown in Table 1(Target weight for shells of special lengths, shapes and designations may be decided upon mutually between the manufacturer of the Hard cellulose Capsule Shells and the user).

Table 1-Average Weight of Hard Cellulose Capsule Shells

Size	Target average weight (mg)
000	163
00	123
0	98
1	78
2	64
3	50
4	40
5	28

Disintegration (2.5.1).Not more than 15 minutes, using discs.

Microbial contamination (2.2.9). Total microbial count not more than 1000 cfu per g, total yeast and mould count not more than 100 cfu per g, 1g is free from *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and 10g is free from *Salmonella* and *Shigella.*

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm) or determined by ICPMS (2.4.42)

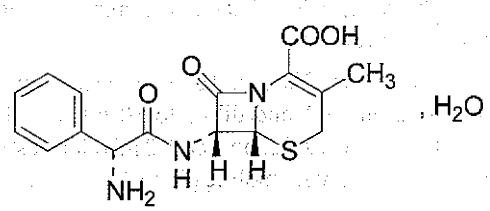
Arsenic (2.3.10). Dissolve 3.3 g of capsule shells in 20 ml of *carbon dioxide free water* and dilute to 50.0 ml with *carbon dioxide free water.* The resulting solution complies with the limit test for arsenic (3 ppm) or determined by ICPMS (2.4.42).

Loss on drying (2.4.19). 3.0 to 9.0 per cent, determined on 1g by drying in an oven at 105° for 4 hours or to constant weight.

Storage. Store protected from moisture at a temperature not exceeding 30°

Labelling The label states (1) the size of the capsule shells; (2) that only permitted colours, if any, have been used; (3) the storage conditions.

Cephalexin



$C_{16}H_{17}N_3O_4S,H_2O$ Mol. Wt. 365.4

Cephalexin is (7*R*)-3-methyl-7-(α-D-phenylglycylamino)-3-cephem-4-carboxylic acid monohydrate.

Cephalexin contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{16}H_{17}N_3O_4S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cephalexin IPRS* or with the reference spectrum of cephalexin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 4.0 to 5.5, determined in a 0.5 per cent w/v solution.

Specific optical rotation (2.4.22). +149° to +158°, determined in a 0.5 per cent w/v solution in *phthalate buffer solution pH 4.4* and in a 2-dm tube.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in mobile phase A and dilute to 50.0 ml with the same solvent.

Reference solution (a). Dissolve 10 mg of *D-phenylglycine* in mobile phase A and dilute to 10.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of *7-amino-desacetoxycephalosporanic acid IPRS* in *phosphate buffer solution pH 7.0* and dilute to 10.0 ml with the same solvent.

Reference solution (c). Dilute 1.0 ml , each of, reference solution (a) and reference solution (b) to 100.0 ml with mobile phase A.

Reference solution (d). Dissolve 10 mg of *dimethylformamide* and 10 mg of *dimethylacetamide* in mobile phase A and dilute to 10 ml with the same solvent. Dilute 1.0 ml to 100.0 ml with mobile phase A.

Reference solution (e). Dilute 1.0 ml of reference solution (c) to 20.0 ml with mobile phase A.

Reference solution (f). Dissolve 10 mg of *cefotaxime sodium IPRS* in mobile phase A and dilute to 10 ml with the same solvent. To 1.0 ml of the solution, add 1.0 ml of the test solution and dilute to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *phosphate buffer solution pH 5.0*, B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
1	98	2
20	70	30
23	98	2
30	98	2

Inject reference solution (c) and (f). In the chromatogram obtained with reference solution (c), the resolution between the peaks due to D-phenylglycine and 7-aminodesacetoxy-cephalosporanic acid is not less than 2.0 and in the chromatogram obtained with reference solution (f) the resolution between the peaks due to cephalexin and cefotaxime is not less than 1.5.

Inject reference solution (c), (d), (e) and the test solution . In the chromatogram obtained with the test solution the area of peak corresponding to D-phenylglycine is not more than the area of the first peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peaks due to dimethylformamide and dimethylacetamide, the area of any other secondary peak is not more than the area of the first peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the sum of the secondary peaks is not more than the three times the area of the first peak in the chromatogram obtained with reference solution (c). Ignore any peak with an area less than the second peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). 4.0 to 8.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *water* and dilute to 100.0 ml with the same solvent.

Reference solution (a). Dissolve 50 mg of *cephalexin monohydrate IPRS* in *water* and dilute to 100.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of *cephradine IPRS* in 20 ml of reference solution (a) and dilute to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 13.6 g per litre solution of *potassium dihydrogen phosphate* and 83 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). In the chromatogram obtained, the resolution between the peaks due to cephalexin and cephradine is not less than 4.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{17}N_3O_4S$.

Storage. Store protected from light at a temperature not exceeding 30°.

Cephalexin Capsules

Cephalexin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous cephalexin, $C_{16}H_{17}N_3O_4S$.

Usual strengths. 250 mg; 500 mg.

Identification

Shake a quantity of the contents of the capsules containing 0.5 g of anhydrous cephalexin with 1 ml of *water* and 1.4 ml of *1 M hydrochloric acid*, filter and wash the filter with 1 ml of *water*. Add slowly to the filtrate a saturated solution of *sodium acetate* until precipitation occurs. Add 5 ml of *methanol*, filter and wash the precipitate with two quantities, each of 1 ml, of *methanol*. The residue after drying at a pressure not exceeding 0.7 kPa complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cephalexin IPRS* or with the reference spectrum of cephalexin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water* freshly prepared by distillation,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 0.8 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *water*. Measure the absorbance of the resulting solution at the maximum at about 261 nm (2.4.7). Calculate the content of $C_{16}H_{17}N_3O_4S$ taking 235 as the specific absorbance at 261 nm.

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{17}N_3O_4S$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF* (such as Analtech plates). Impregnate the plate by development with a 5 per cent v/v solution of *n-tetradecane* in *hexane*. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation.

Mobile phase. A mixture of 3 volumes of *acetone*, 80 volumes of a 7.2 per cent w/v solution of *disodium hydrogen orthophosphate* and 120 volumes of a 2.1 per cent w/v solution of *citric acid*.

Test solution. Shake a quantity of the contents of the capsules containing about 0.25 g of anhydrous cephalexin with 10 ml of *2 M hydrochloric acid*, filter and use the filtrate.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with *2 M hydrochloric acid*.

Reference solution (b). A 0.025 per cent w/v solution of *7-amino-desacetoxycephalosporanic acid IPRS* in *2 M hydrochloric acid*.

Reference solution (c). A 0.025 per cent w/v solution of *DL-phenylglycine* in *2 M hydrochloric acid*.

Reference solution (d). A solution containing 2.5 per cent w/v of *cephalexin IPRS* and 0.025 per cent w/v, each of, *7-amino-desacetoxycephalosporanic acid IPRS* and *DL-phenylglycine* in *2 M hydrochloric acid*.

Apply to the plate 5 µl of each solutions. After development, dry the plate at 90° for 3 minutes, spray the hot plate with a 0.1 per cent w/v solution of *ninhydrin* in the mobile phase, heat the plate at 90° for 15 minutes and allow to cool. In the chromatogram obtained with the test solution any spot corresponding to 7-aminodesacetoxy-cephalosporanic acid is not more intense than the spot in the chromatogram obtained with reference solution (b) (1 per cent), any spot corresponding to *DL-phenylglycine* is not more intense than the spot in the chromatogram obtained with reference solution (c) (1.0 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows three clearly separated spots.

Water (2.3.43). Not more than 10.0 per cent, determined on 0.3 g of the contents of the capsules.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered mixed contents of 20 capsules containing about 0.25 g of anhydrous cephalexin with 100.0 ml of *water* for 30 minutes, add sufficient amount of *water* to produce 250.0 ml and filter. Dilute 25.0 ml of the filtrate to 50.0 ml with *water*.

Reference solution (a). A 0.05 per cent w/v solution of *cephalexin IPRS* in *water*.

Reference solution (b). A solution containing 0.01 per cent w/v, each of, *cephalexin IPRS* and *cephradine IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C 18),
- mobile phase: a mixture of 2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 13.6 g per litre solution of *potassium dihydrogen phosphate* and 83 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to cephalexin and cephradine is at least 4.0.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{17}N_3O_4S$ in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cephalexin.

Cephalexin Oral Suspension

Cephalexin Dry Syrup; Cephalexin Mixture

Cephalexin Oral Suspension is a mixture of Cephalexin with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Cephalexin Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of $C_{16}H_{17}N_3O_4S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cephalexin, $C_{16}H_{17}N_3O_4S$.

Usual strengths. 125 mg; 250 mg of anhydrous cephalexin per 5 ml.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

B. Weigh a quantity containing 0.1 g of anhydrous cephalexin, shake with 20 ml of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in the minimum volume of a 1 per cent v/v solution of *glacial acetic acid*, decolorise if necessary by the addition of sufficient *decolorising charcoal*, shake and filter. To 0.25 ml of the resulting solution add 0.1 ml of a 1 per cent w/v solution of *cupric sulphate* and 0.05 ml of *2 M sodium hydroxide*; an olive-green colour is produced.

Tests

Other tests. Comply with the tests stated under Oral liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake an accurately weighed quantity of the oral suspension containing about 0.25 g of anhydrous cephalexin with 100.0 ml of *water* for 30 minutes, add sufficient of *water* to produce 250.0 ml and filter. Dilute 25.0 ml of the filtrate to 50.0 ml with *water*.

Reference solution (a). A 0.05 per cent w/v solution of *cephalexin IPRS* in *water*.

Reference solution (b). A solution containing 0.01 per cent w/v, each of, *cephalexin IPRS* and *cephradine IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C 18),
- mobile phase: a mixture of 2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 13.6 g per litre solution of *potassium dihydrogen phosphate* and 83 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to cephalexin and cephradine is at least 4.0.

Inject reference solution (a). The relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{16}H_{17}N_3O_4S$, weight in volume.

Repeat the procedure using a portion of the suspension that has been stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cephalexin.

Cephalexin Tablets

Cephalexin Tablets contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous cephalexin, $C_{16}H_{17}N_3O_4S$.

Usual strengths. 250 mg; 500 mg.

Identification

Remove any coating. Shake a quantity of the powdered tablet cores containing 0.5 g of anhydrous cephalexin with 1 ml of *water* and 1.4 ml of *1 M hydrochloric acid*, add 0.1 g of *decolorising charcoal*, shake, filter and wash the filter with 1 ml of *water*. Add slowly to the filtrate a saturated solution of *sodium acetate* until precipitation occurs. Add 5 ml of *methanol*, filter and wash the precipitate with two quantities, each of 1 ml, of *methanol*. The residue, after drying at a pressure not exceeding 0.7 kPa, complies with the following test.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cephalexin IPRS* or with the reference spectrum of cephalexin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water* freshly prepared by distillation,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *water*. Measure the absorbance of the resulting solution at the maximum at about 261 nm (2.4.7). Calculate the content of $C_{16}H_{17}N_3O_4S$ taking 235 as the specific absorbance at 261 nm.

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{17}N_3O_4S$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF*. Impregnate the plate by development with a 5 per cent v/v solution of *n-tetradecane* in hexane. Allow the solvent to evaporate and carry out the chromatography in the same direction as the impregnation.

Mobile phase. A mixture of 3 volumes of *acetone*, 80 volumes of a 7.2 per cent w/v solution of *disodium hydrogen orthophosphate* and 120 volumes of a 2.1 per cent w/v solution of *citric acid*.

Test solution. Shake a quantity of the powdered tablets containing 0.25 g of anhydrous cephalexin with 10 ml of *2 M hydrochloric acid*, filter and use the filtrate.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with *2 M hydrochloric acid*.

Reference solution (b). A 0.025 per cent w/v solution of *7-aminodesacetoxycephalosporanic acid IPRS* in *2 M hydrochloric acid*.

Reference solution (c). A 0.025 per cent w/v solution of DL-*phenylglycine* in *2 M hydrochloric acid*.

Reference solution (d). A solution containing 2.5 per cent w/v of *cephalexin IPRS* and 0.025 per cent w/v, each of, *7-amino-desacetoxycephalosporanic acid IPRS* and DL-*phenylglycine* in *2 M hydrochloric acid*.

Apply separately to the plate 5 µl of each solution. After development, dry the plate at 90° for 3 minutes, spray the hot plate with a 0.1 per cent w/v solution of *ninhydrin* in the mobile phase, heat the plate at 90° for 15 minutes and allow to cool. In the chromatogram obtained with the test solution any spot corresponding to 7-aminodesacetoxycephalosporanic acid is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent), any spot corresponding to DL-*phenylglycine* is not more intense than the spot in the chromatogram obtained with reference solution (c) (1.0 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows three clearly separated spots.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.25 g of anhydrous cephalexin with 100.0 ml of *water* for 30 minutes, add sufficient amount of

water to produce 250.0 ml and filter. Dilute 25.0 ml of the filtrate to 50.0 ml with *water*.

Reference solution (a). A 0.05 per cent w/v solution of *cephalexin IPRS* in *water*.

Reference solution (b). A solution containing 0.01 per cent w/v, each of, *cephalexin IPRS* and *cephradine IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C 18),
- mobile phase: a mixture of 2 volumes of *methanol*, 5 volumes of *acetonitrile*, 10 volumes of a 13.6 g per litre solution of *potassium dihydrogen phosphate* and 83 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to cephalexin and cephradine is at least 4.0.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

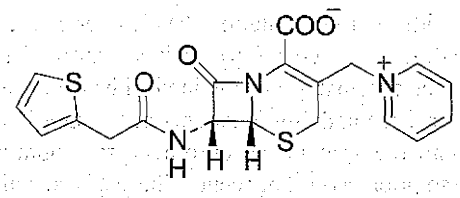
Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{17}N_3O_4S$ in the tablets.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cephalexin. If the tablets are dispersible, the label also states that the tablets should be dispersed in water immediately before use.

Cephaloridine



$C_{19}H_{17}N_3O_4S_2$ Mol. Wt. 415.5

Cephaloridine is (7*R*)-3-(1-pyridiniomethyl)-7-[(2-thienyl)-acetamido]-3-cephem-4-carboxylate (α-form or δ -form).

Cephaloridine contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{19}H_{17}N_3O_4S_2$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cephaloridine (α-form) IPRS* or *cephaloridine (δ-form) IPRS* or with the reference spectrum of cephaloridine (α-form) *or* cephaloridine (δ -form).

B. Mix 20 mg with a few drops of an 80 per cent v/v solution of *sulphuric acid* containing 1 per cent v/v of *nitric acid;* a bluish-green colour is produced.

C. To a 0.5 per cent w/v solution add 1 ml of *chloramine solution* and 2 ml of *0.1 M sodium hydroxide*; a dull red colour is produced which persists for 1 minute.

D. It gives the reactions of penicillins and cephalosporins (2.3.1).

Tests

Appearance of solution (2.4.1). Solution A is clear.

pH (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution (solution A) prepared by dissolving in *carbon dioxide-free water,* warming to 30° and cooling to 20°.

Specific optical rotation (2.4.22). +46.0° to +50.0°, determined at 25° in a 1.0 per cent w/v solution.

Light absorption. When examined in the range 230 nm to 360 nm (2.4.7) a 0.0012 per cent w/v solution shows absorption maxima at about 240 nm and 255 nm; absorbance at the maximum at about 240 nm, 0.43 to 0.48. The ratio of the absorbance at the maximum at about 240 nm to that at about 255 nm is not more than 1.10.

Pyridine. Dissolve about 25 mg in 10 ml of *water* and add 2.5 ml of a buffer solution prepared by adjusting a 5 per cent w/v solution of *disodium hydrogen phosphate* to pH 6.0 with *phosphoric acid* and adding 1 per cent v/v of *aniline*. Add 1.25 ml of a solution prepared by decolorising a 0.5 per cent v/v solution of *bromine* with *potassium cyanide solution*, shaking and allowing to stand for 2 minutes, and sufficient *water* to produce 25 ml and allow to stand for 25 minutes. Measure the absorbance of the resulting solution at the maximum at about 462 nm, using as the blank a solution prepared in a similar manner but omitting the substance under examination (2.4.7). The absorbance is not more than that of a solution prepared by treating 2.5 ml of a 0.005 per cent w/v solution of *pyridine* in a similar manner.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent w/w (α-form) and not more than 3.0 per cent w/w (δ-form), determined on 0.25 g. Use as the solvent a mixture of equal volumes of dehydrated methanol and dehydrated pyridine in place of methanol.

Assay. Weigh accurately about 60 mg and dissolve in sufficient *water* to produce 50.0 ml. Transfer 10.0 ml to a stoppered flask, add 5 ml of *1 M sodium hydroxide* and allow to stand for 20 minutes. Add 20 ml of a buffer solution containing 35.0 per cent w/v of *sodium acetate* and 42.4 per cent v/v of *glacial acetic acid*, 5 ml of *1 M hydrochloric acid* and 25.0 ml of *0.01 M iodine*, close the flask with a wet stopper and allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with *0.02 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. To a further 10.0 ml of the solution add 20 ml of the buffer solution and 25.0 ml of *0.01 M iodine*, allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with *0.02 M sodium thiosulphate* using *starch* solution, added towards the end of the titration, as indicator. The difference between the titrations represents the volume of *0.01 M iodine* equivalent to the cephaloridine present. Calculate the content of $C_{19}H_{17}N_3O_4S_2$ from the difference obtained by simultaneously carrying out the Assay using *cephaloridine (δ-form) IPRS* instead of the substance under examination.

Cephaloridine intended for use in the manufacture of parenteral preparations complies with the following additional tests.

Pyrogens. Complies with the test for pyrogens (2.2.8), using not less than 50 mg per kg of the rabbit's weight, dissolved in 1 ml of Water for injections.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture in a refrigerator (8° to 15°). If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) whether the contents are Cephaloridine (α-form) or Cephaloridine (δ-form); (2) whether or not it is intended for use in the manufacture of injectable preparations.

Cephaloridine Injection

Cephaloridine Injection is a sterile material consisting of Cephaloridine with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of Water for Injections immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cephaloridine Injection contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous cephaloridine, $C_{19}H_{17}N_3O_4S_2$.

Usual strengths. 250 mg; 500 mg; 1 g.

Description. A white or almost white, crystalline powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cephaloridine (α-form) IPRS* or *cephaloridine (δ-form) IPRS* or with the reference spectrum of cephaloridine (α-form) or cephaloridine (δ-form).

B. Mix 20 mg with a few drops of an 80 per cent v/v solution of *sulphuric acid* containing 1 per cent v/v of *nitric acid;* a bluish-green colour is produced.

C. To a 0.5 per cent w/v solution add 1 ml of *chloramine solution* and 2 ml of *0.1 M sodium hydroxide*; a dull red colour is produced which persists for 1 minute.

D. It gives the reactions of penicillins and cephalosporins (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1).

pH (2.4.24). 4.0 to 6.0, determined in a 10.0 per cent w/v solution (solution A) prepared by dissolving in *carbon dioxide-free water,* warming to 30° and cooling to 20°.

Specific optical rotation (2.4.22). +46.0° to +50.0°, determined at 25° in a 1.0 per cent w/v solution.

Light absorption. When examined in the range 230 nm to 360 nm (2.4.7) a 0.0012 per cent w/v solution shows absorption maxima at about 240 nm and 255 nm; absorbance at the maximum at about 240 nm, 0.43 to 0.48. The ratio of the absorbance at the maximum at about 240 nm to that at about 255 nm is not more than 1.10.

Pyridine. Dissolve about 25 mg in 10 ml of *water* and add 2.5 ml of a buffer solution prepared by adjusting a 5 per cent w/v solution of *disodium hydrogen phosphate* to pH 6.0 with *phosphoric acid* and adding 1 per cent v/v of *aniline*. Add 1.25 ml of a solution prepared by decolorising a 0.5 per cent v/v solution of *bromine* with *potassium cyanide solution*, shaking and allowing to stand for 2 minutes, and sufficient *water* to produce 25 ml and allow to stand for 25 minutes. Measure the absorbance of the resulting solution at the maximum at about 462 nm, using as the blank a solution

prepared in a similar manner but omitting the substance under examination (2.4.7). The absorbance is not more than that of a solution prepared by treating 2.5 ml of a 0.005 per cent w/v solution of *pyridine* in a similar manner.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent w/w (α-form) and not more than 3.0 per cent w/w (δ-form), determined on 0.25 g. Use as the solvent a mixture of equal volumes of *dehydrated methanol* and dehydrated pyridine in place of *methanol*.

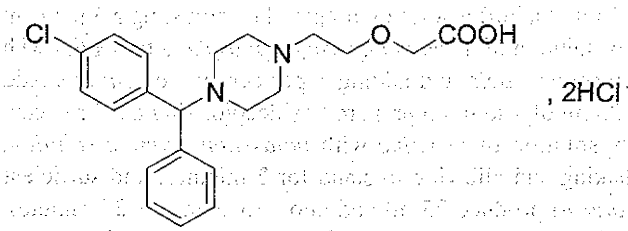
Assay. Determine the weight of the contents of 10 containers Weigh accurately a quantity of the mixed contents of the 10 containers containing about 60 mg of cephaloridine and dissolve in sufficient *water* to produce 50.0 ml. Transfer 10.0 ml to a stoppered flask, add 5 ml of *1 M sodium hydroxide* and allow to stand for 20 minutes. Add 20 ml of a buffer solution containing 35.0 per cent w/v of *sodium acetate* and 42.4 per cent v/v of *glacial acetic acid*, 5 ml of *1 M hydrochloric acid* and 25.0 ml of *0.01 M iodine*, close the flask with a wet stopper and allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with *0.02 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. To a further 10.0 ml of the solution add 20 ml of the buffer solution and 25.0 ml of *0.01 M iodine*, allow to stand for 3 hours in a water-bath at 30°, protected from light. Titrate the excess of iodine with *0.02 M sodium thiosulphate* using *starch* solution, added towards the end of the titration, as indicator. The difference between the titrations represents the volume of *0.01 M iodine* equivalent to the cephaloridine present. Calculate the content of $C_{19}H_{17}N_3O_4S_2$ from the difference obtained by simultaneously carrying out the Assay using *cephaloridine (δ-form) IPRS* instead of the substance under examination.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states (1) the weight of Cephaloridine contained in the sealed container; (2) whether the contents are Cephaloridine (α-form) or Cephaloridine (δ-form).

Cetirizine Hydrochloride

Cetirizine Dihydrochloride



$C_{21}H_{25}ClN_2O_3,2HCl$ Mol. Wt. 461.8

Cetirizine Hydrochloride is [2-[4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy]acetic acid dihydrochloride.

Cetirizine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{25}ClN_2O_3,2HCl$, calculated on the dried basis.

Category. Antihistaminic.

Description. A white or almost white powder.

Identification

Test B and C may be omitted if tests A and D are carried out. Tests A may be omitted if test B, C and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cetirizine hydrochloride IPRS* or with the reference spectrum of cetirizine hydrochloride.

B. Dissolve 20.0 mg in 50 ml of a 1.03 per cent w/v solution of *hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of the solution to 100.0 ml with the acid.

When examined in the range 210 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum at about 231 nm. The specific absorbance at 231 nm is 359 to 381.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *ammonia, 10* volumes of *methanol* and 90 volumes of *dichloromethane*.

Test solution. Dissolve 10 mg of the substance under examination in *water* and dilute to 5 ml with the same solvent.

Reference solution (a). Dissolve 10 mg of *cetirizine hydrochloride IPRS* in *water* and dilute to 5.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of *chlorphenamine maleate IPRS* in *water* and dilute to 5.0 ml with the same solvent. To 1 ml of the solution add 1 ml of reference solution (a).

Apply to the plate 5 µl of each solution. After development, dry in a current of cold air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

D. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

pH (2.4.24). 1.2 to 1.8, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.02 per cent w/v, each of, *cetirizine dihydrochloride IPRS* and *(RS)-1-[(4-chlorophenyl)phenylmethyl]piperazine IPRS (cetirizine impurity A)* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 2.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with silica gel (5 µm),
- mobile phase: a mixture of 0.4 volume of *dilute sulphuric acid,* 6.6 volumes of *water* and 93 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm.
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cetirizine and impurity A is not less than 2 and the tailing factor is not more than 2.0 for cetirizine peak.

Inject reference solution (b) and the test solution. Run the chromatogram for 3 times the retention time of cetirizine. In the chromatogram obtained with the test solution, the area of any impurity peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all such peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° to 105°.

Assay. Weigh accurately about 0.1 g, dissolve in 70 ml of a mixture of 30 volumes of *water* and 70 volumes of *acetone.* Titrate with *0.1 M sodium hydroxide* to the second point of inflexion. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01539 g of $C_{21}H_{27}Cl_3N_2O_3$.

Storage. Store protected from light.

Cetirizine Syrup

Cetirizine Oral Liquid

Cetirizine Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cetirizine hydrochloride $C_{21}H_{25}ClN_2O_3,2HCl$.

Usual strength. 5 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 5.5.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water* and 40 volumes of *acetonitrile.*

Test solution. Weigh accurately a quantity of the syrup containing 5 mg of Cetirizine Hydrochloride, dissolve in 100.0 ml of the solvent mixture and filter.

Reference solution. A 0.005 per cent w/v solution of *cetirizine dihydrochloride IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *0.0025 M 1-heptane sulphonic acid* prepared by dissolving 0.55 g of *1-heptane sulphonic acid sodium* in 1000 ml of *water,* adjusted to pH 3.5 with *0.1 M sulphuric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml (2.4.29) of the syrup and calculate the content of $C_{21}H_{25}ClN_2O_3,2HCl$, weight in volume.

Storage. Store protected from light, at a temperature not exceeding 30°.

Cetirizine Tablets

Cetirizine Hydrochloride Tablets

Cetirizine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cetirizine hydrochloride, $C_{21}H_{25}ClN_2O_3,2HCl$.

Usual strengths. 10 mg; 20 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the dissolution medium if necessary, at the maximum at about 230 nm (2.4.7). Calculate the content of $C_{21}H_{25}ClN_2O_3,2HCl$ in the medium from the absorbance obtained from a solution of known concentration of *cetirizine hydrochloride IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{25}ClN_2O_3,2HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Cetirizine Hydrochloride, add 50 ml of the mobile phase, mix and dilute to 100 ml with the mobile phase.

Reference solution (a). A solution containing 0.02 per cent w/v, each of, *cetirizine hydrochloride IPRS* and *(RS)-1-[(4-chlorophenyl)phenylmethyl]piperazine IPRS (cetirizine impurity A)* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with silica gel (5 µm),
- mobile phase: a mixture of 0.4 volume of *dilute sulphuric acid,* 6.6 volumes of *water* and 93 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cetirizine and cetirizine impurity A is not less than 2.0 and the tailing factor is not more than 2.0 for cetirizine peak.

Inject reference solution (b) and the test solution. Run the chromatogram 3 times the retention time of cetirizine. In the chromatogram obtained with the test solution the area of any impurity peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all such peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

Test solution. Disperse 1 tablet in the mobile phase, mix and dilute to 100.0 ml with the mobile phase, filter. Dilute 5.0 ml of the solution to 10.0 ml with mobile phase.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 25 mg of Cetirizine Hydrochloride, add the mobile phase, mix and dilute to 50.0 ml with the mobile phase, filter. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase.

Reference solution. A 0.05 per cent w/v solution of *cetirizine hydrochloride IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: dissolve 0.19 g of *heptane sulphonic acid sodium salt* in 300 ml *water* add 700 ml *acetonitrile* and mix. Adjust pH to 3.2 with *0.05 M sulphuric acid*, filter,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{25}ClN_2O_3,2HCl$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Cetostearyl Alcohol

Cetostearyl Alcohol is a mixture of solid aliphatic alcohols consisting chiefly of stearyl and cetyl alcohols.

Cetostearyl Alcohol contains not less than 40.0 per cent of stearyl alcohol and sum of stearyl alcohol and cetyl alcohol is not less than 90.0 per cent.

Category. Pharmaceutical aid (ointment base).

Description. A white or pale yellow, wax like mass, plates, flakes or granules.

Identification

In the Assay, the two principal peaks in the chromatogram obtained with the test solution corresponds to the principal peaks in the chromatogram obtained with the reference solution.

Tests

Melting range (2.4.21). 47° to 56°, determined by Method II. Introduce the substance under examination into the capillary tubes and allow to stand at 2° to 8° for 12 hours before carrying out the determination.

Appearance of solution. Dissolve 0.5 g in 20 ml of boiling *ethanol (95 per cent)*. The solution is clear (2.4.1) and not more intensely coloured than reference solution BS6 (2.4.1).

Acid value (2.3.23). Not more than 1.0.

Hydroxyl value (2.3.27). 208 to 228.

Saponification value (2.3.37). Not more than 2.0.

Iodine value (2.3.28). Not more than 3.0, determined by Method B in a 8.0 per cent w/v solution in *chloroform*.

Hydrocarbons. Dissolve 2.0 g in 100 ml of *light petroleum (40° to 60°)*, warming slightly if necessary, and transfer the solution to a column (25 cm x 10 mm) of *anhydrous alumina* which has been slurried with *light petroleum (40° to 60°)*. Elute with two portions, each of 50 ml, of *light petroleum (40° to 60°)* into a flask, remove the light petroleum and dry at 80°; the residue weighs not more than 30 mg.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of the *ethanol (95 per cent)*.

Reference solution. A solution containing 0.6 per cent w/v of *cetyl alcohol IPRS* and 0.4 per cent w/v of *stearyl alcohol IPRS* in *ethanol (95 per cent)*. Dilute 1.0 ml of the solution to 10.0 ml with the same solvent.

Chromatographic system

- a capillary column 30 m x 0.32 mm packed with poly(dimethyl)siloxane (1 µm),
- temperature:

column	time (min)	temperature (°)
	0-20	150-250
	20-40	250

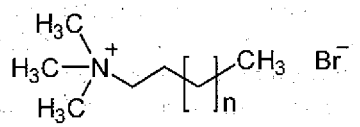
- Inlet port and detector at 250°,
- flame ionization detector,
- flow rate: 1ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks due to cetyl alcohol and stearyl alcohol is not less than 5.0.

Inject the test solution.

Calculate the content of cetyl alcohol, $C_{16}H_{34}O$ and stearyl alcohol, $C_{18}H_{38}O$ by area normalisation.

Cetrimide



Cetrimide consists of trimethyltetradecylammonium bromide (n = 11) with small amounts of trimethylhexadecylammonium bromide (n = 13) and trimethyldodecylammonium bromide (n = 9).

Cetrimide contains not less than 96.0 per cent and not more than 101.0 per cent of alkyltrimethylammonium bromides, calculated as $C_{17}H_{38}BrN$ (336.4) on the dried basis.

Category. Pharmaceutical aid; bactericide.

Description. A white or creamy-white, voluminous, free-flowing powder.

Identification

A. To 10 ml of a 1 per cent w/v solution add 2 ml of *potassium ferricyanide solution*; a yellow precipitate is produced.

B. To 10 ml of a 1 per cent w/v solution add 2 ml of a 10 per cent w/v solution of *sodium silicate*; a white flocculent precipitate is produced.

C. To 10 ml of a 1 per cent w/v solution add 2 ml of *dilute nitric acid*; a yellow precipitate is produced. Filter and to the filtrate add 2 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; a yellow precipitate is produced.

D. Dissolve 0.25 g in sufficient *ethanol (95 per cent)* to produce 25 ml. Absorbance of the resulting solution between 260 nm and 280 nm (2.4.7) is not more than 0.05.

Tests

Appearance of solution. A 2.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. Dissolve 1.0 g in 50 ml of *water* and add 2 drops of *bromocresol purple solution*. Not more than 0.1 ml of either *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the solution.

Amine salts. Carry out the Assay described below using a further 25.0 ml of the original solution and 10 ml of *0.1 M hydrochloric acid* instead of the *0.1 M sodium hydroxide*. The difference between the volume of *0.05 M potassium iodate* required in the titration and that required in the Assay is not more than 1.0 ml for each g of the substance used.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh accurately about 2.0 g and dissolve in sufficient *water* to produce 100.0 ml. Transfer 25.0 ml of the solution to a separator, add 25 ml of *chloroform*, 10 ml of *0.1 M sodium hydroxide* and 10.0 ml of a freshly prepared 5.0 per cent w/v solution of *potassium iodide*. Shake well, allow to separate, and discard the chloroform layer. Shake the aqueous solution with three quantities, each of 10 ml, of *chloroform* and discard the *chloroform solution*. Add 40 ml of *hydrochloric acid*, allow to cool and titrate with *0.05 M potassium iodate* until the deep brown colour is almost discharged. Add 2 ml of *chloroform* and continue the titration, with shaking, until the chloroform layer no longer changes colour. Carry out a blank titration on a mixture of 20 ml of *water*, 10.0 ml of the freshly prepared *potassium iodide solution* and 40 ml of *hydrochloric acid*. The difference between the titrations represents the amount of potassium iodate required.

1 ml of *0.05 M potassium iodate* is equivalent to 0.03364 g of $C_{17}H_{38}BrN$.

Cetrimide Cream

Cetrimide	5 g
Cetostearyl Alcohol	50 g
Liquid Paraffin	500 g
Purified Water	1000 g

Melt the Cetostearyl Alcohol and heat with the Liquid Paraffin to about 60°. Dissolve the Cetrimide in sufficient Purified Water to produce about 450 g. Add the aqueous solution to the oily phase when both are at about 60° and mix. Stir gently until cool, add sufficient of the Purified Water to produce 1000 g and mix.

Cetrimide Cream contains not less than 88.0 per cent and not more than 106.0 per cent w/w of the stated amount of cetrimide, $C_{17}H_{38}BrN$.

Identification

Mix 1 g with 50 ml of *water*. The diluted cream complies with the following tests:

A. To 10 ml, add 2 ml of *potassium ferricyanide* solution; a yellow precipitate is produced.

B. Shake 3 ml of *water* with 1 ml of *1 M sulphuric acid*, 2 ml of *chloroform* and 0.5 ml of *methyl orange solution*. Add 2 ml of the diluted cream shake and allow to separate; a yellow colour develops in the chloroform layer.

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Weigh a quantity of the cream containing 5 mg of Cetrimide add 10 ml of hot *water* and shake gently until dispersed. Add 5 ml of *1 M sulphuric acid*, 20 ml of *chloroform* and 0.25 ml of *dimethyl yellow solution* and titrate with *0.001 M dioctyl sodium sulphosuccinate*.

1 ml of *0.001 M dioctyl sodium sulphosuccinate* is equivalent to 0.0003364 g of $C_{17}H_{38}BrN$.

Labelling. The label states the strength as the percentage w/w of Cetrimide.

Cetrimide Emulsifying Ointment

Cetrimide Emulsifying Ointment is an ointment containing cetrimide in a suitable base.

Cetrimide Emulsifying Ointment contains not less than 2.5 per cent and not more than 3.3 per cent w/w of the stated amount of cetrimide, $C_{17}H_{38}BrN$.

Tests

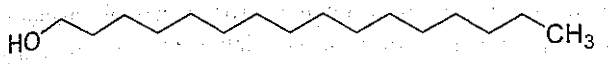
Other tests. Comply with the tests stated under Ointments.

Assay. To 0.3 g in a stoppered cylinder, add 10 ml of hot *water* and shake until the solid is dispersed. Add 5 ml of *1 M sulphuric acid*, 20 ml of *chloroform* and 1 ml of *dimethyl yellow and oracet blue B solution* and titrate with *0.001 M sodium dodecyl sulphate*.

1 ml of *0.001 M sodium dodecyl sulphate* is equivalent to 0.3364 mg of $C_{17}H_{38}BrN$.

Cetyl Alcohol

Palmityl Alcohol; *n*-Hexadecyl Alcohol; 1-Hexadecanol



Cetyl Alcohol is a mixture of solid alcohols consisting mainly of 1-hexadecanol, $C_{16}H_{34}O$.

Cetyl Alcohol contains not less than 95.0 per cent of $C_{16}H_{34}O$.

Category. Pharmaceutical aid (stiffening, emulsifying and tablet coating agent).

Description. A white, unctuous mass, powder, flakes or granules.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (a).

Tests

Melting range (2.4.21). 46° to 52°, determined by Method II. Introduce the substance under examination into the capillary tubes and allow to stand at 2° to 8° for 12 hours before carrying out the determination.

Appearance of solution. Dissolve 0.5 g in boiling *ethanol (95 per cent)*, cool and dilute to 20 ml with the same solvent. The resulting solution is clear (2.4.1) and not more intensely coloured than reference solution BS6 (2.4.1).

Acid value (2.3.23). Not more than 1.0.

Hydroxyl value (2.3.27). 218 to 238.

Saponification value (2.3.37). Not more than 2.0.

Iodine value (2.3.28). Not more than 2.0, determined by Method B in a 8.0 per cent w/v solution in *chloroform*.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of the *ethanol (95 per cent)*.

Reference solution (a). Dissolve 50 mg of *cetyl alcohol IPRS* in 5.0 ml of the *ethanol (95 per cent)*.

Reference solution (b). Dissolve 50 mg of *stearyl alcohol IPRS* in 10.0 ml of the *ethanol (95 per cent)*.

Reference solution (c). Mix 1.0 ml, each of, reference solution (a) and (b) and dilute to 10.0 with the *ethanol (95 per cent)*.

Chromatographic system

- a capillary column 30 m x 0.32 mm packed with poly(dimethyl)siloxane (1 µm),
- temperature:

column	time (min)	temperature (°)
	0 - 20	150 - 250
	20 - 40	250

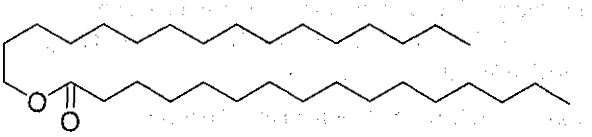
- inlet port and detector at 250°,
- flame ionization detector,
- flow rate: 1 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of reference solution (c). The test is not valid unless the resolution between the peaks due to cetyl alcohol and stearyl alcohol is not less than 5.0.

Inject reference solution (a) and the test solution.

Calculate the content of cetyl alcohol, $C_{16}H_{34}O$.

Cetyl Palmitate



$C_{32}H_{64}O_2$ Mol. Wt. 480.9

Cetyl Palmitate is hexadecyl palmitate.

Cetyl Palmitate is a mixture of C_{14} to C_{18} esters of lauric acid (dodecanoic), myristic acid (tetradecanoic), palmitic acid (hexadecanoic) and stearic acid (octadecanoic) (Cetyl esters wax).

Cetyl Palmitate contains not less than 10.0 per cent and not more than 20.0 per cent for cetyl palmitate 15; not less than 60.0 per cent and more than 70.0 per cent for cetyl palmitate 65 and not less than 90.0 per cent for Cetyl palmitate 95.

Category. Pharmaceutical aid.

Description. A white or almost white, waxy plates, flakes or powder.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (a) and (b).

Tests

Appearance of solution. A 20.0 per cent w/v solution in *dichloromethane*, is not more intensely coloured than reference solution YS6 (2.4.1).

Melting point (2.4.21). About 45° for Cetyl palmitate 15 and Cetyl palmitate 65 and about 52° for Cetyl palmitate 95.

Acid value (2.3.23). Not more than 4.0. Dissolve 10 g in 50 ml of the solvent mixture described by heating under reflux on a water-bath for 5 minutes.

Hydroxyl value (2.3.27). Not more than 20.

Saponification value (2.3.37). 105 to 120. Heat under reflux for 2 hours.

Iodine value (2.3.28). Not more than 2.0.

Alkaline substances. Dissolve 2.0 g with gentle heating in a mixture of 1.5 ml of *ethanol (95 per cent)* and 3 ml of *toluene*. Add 0.05 ml of a 4 per cent w/v solution of *bromophenol blue* in *ethanol (95 per cent)*. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to yellow.

Nickel. Dissolve 10.0 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and add 10 ml of *6 M ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *ethanol (95 per cent)*. Mix, dilute to 50 ml with *water* and allow to stand for 5 minutes; any colour produced is not more intense than that produced by treating in the same manner and at the same time 1.0 ml of *nickel standard solution (10 ppm Ni)* diluted to 20 ml with *water* (1 ppm).

Total ash (2.3.19). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.3 per cent w/w, determined on 1.0 g using a mixture of equal volumes of *anhydrous methanol* and *dichloromethane*.

Assay. Determine by gas chromatography (2.4.13), using area normalization method.

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of *hexane*.

Reference solution (a). Dissolve 25 mg of *cetyl palmitate 95 IPRS* in 25.0 ml of *hexane*.

Reference solution (b). Dissolve 25 mg of *cetyl palmitate 15 RS* in 25.0 ml of *hexane*.

Chromatographic system

- a capillary column 10 m x 0.53 mm, packed with poly(dimethyl)siloxane (film thickness 2.65 µm),
- temperature:
  column 100° to 300° @ 10° per minute,
  inlet port and detector 350°,
- a flame ionisation detector,
- flow rate: 6.5 ml per minute, using nitrogen as the carrier gas.

The relative retention time with reference to cetyl palmitate is about 9 minutes, for cetyl alcohol is about 0.3, for palmitic acid is about 0.4, for lauric ester is about 0.8, for myristic ester is about 0.9 and for stearic ester is about 1.1

Inject 1 µl of reference solution (b). The test is not valid unless the resolution between the peaks due to cetyl palmitate and cetyl stearate is not less than 1.5.

Inject 1 µl each of reference solution (a), (b) and the test solution.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the type of cetyl palmitate.

Activated Charcoal

Decolorising Charcoal

Activated Charcoal is obtained from vegetable matter by suitable carbonisation processes intended to confer a high adsorbing power.

Category. Adsorbent.

Description. A light, black powder, free from grittiness.

Identification

A. When heated to redness, burns slowly without flame.

B. Complies with the test for Adsorbing power.

Tests

Acidity or alkalinity. Boil 2.0 g with 40 ml of *water* for 5 minutes. Cool, restore to the original volume with *carbon dioxide-free water* and filter, discarding the first 20 ml of the filtrate. To 10 ml of the filtrate add 0.25 ml of *bromothymol blue solution* and 0.25 ml of *0.02 M sodium hydroxide*. The solution is blue and not more than 0.75 ml of *0.02 M hydrochloric acid* is required to change the colour to yellow.

Acid-soluble substances. Boil 1.0 g with a mixture of 20 ml of *water* and 5 ml of *hydrochloric acid* for 5 minutes, filter whilst hot and collect the filtrate in a previously weighed porcelain crucible, wash the residue with 10 ml of hot *water*, adding the washing to the filtrate. To the combined filtrate and washing add 1 ml of *hydrochloric acid*, evaporate to dryness and ignite gently to constant weight; the residue weighs not more than 30 mg.

Ethanol-soluble substances. Boil 2.0 g with 50 ml of *ethanol (95 per cent)* under a reflux condenser for 10 minutes. Filter immediately, cool and adjust the volume to 50 ml with *ethanol (95 per cent)*. The filtrate is not more intensely coloured than reference solution BYS6 or YS6 (2.4.1). Evaporate 40 ml of the filtrate to dryness; the residue, after drying to constant weight at 105°, weighs not more than 8 mg.

Alkali-soluble coloured matter. Boil 0.25 g with 10 ml of *2 M sodium hydroxide* for 1 minute, cool and filter; the filtrate, when diluted to 10 ml with *water*, is not more intensely coloured than reference solution GYS4, (2.4.1).

Chlorides (2.3.12). Boil 3.0 g with 75 ml of *water* for 5 minutes, cool. Dilute to 100.0 ml with *water* and filter; 6.0 ml of the filtrate complies with the limit test for chlorides. (0.14 per cent).

Sulphates (2.3.17). 10.0 ml of the filtrate obtained in the test for Chloride complies with the limit test for sulphates (500 ppm).

Sulphide. Heat 1.0 g with a mixture of 20 ml of *water* and 5 ml of *7 M hydrochloric acid* to boiling; the fumes evolved do not turn *lead acetate paper* brown.

Uncarbonised constituents. Boil 0.25 g with 10 ml of *1 M sodium hydroxide* for few seconds and filter; the filtrate is colourless.

Copper. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 325.0 nm using an air-acetylene flame and a solution prepared in the following manner. Boil 2.0 g with 50 ml of *2 M hydrochloric acid* under a reflux condenser for 1 hour. Filter, wash the filter with *2 M hydrochloric acid* and evaporate the combined filtrate to dryness on a water-bath. Dissolve the residue in sufficient *0.1 M hydrochloric acid* to produce 50.0 ml. Use *copper solution AAS*, suitably diluted with *0.1 M hydrochloric acid*, for preparing the standard solution (25 ppm).

Reserve the solution for the tests for Lead and Zinc.

Lead. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 283.3 nm or 217.0 nm using an air-acetylene flame. Use the solution prepared in the test for Copper as the test solution and *lead solution AAS*, suitably diluted with *0.1 M hydrochloric acid*, for preparing the standard solution (10 ppm).

Zinc. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 214.0 nm using an air-acetylene flame. Use the solution prepared in the test for Copper as the test solution and *zinc solution AAS*, suitably diluted with *0.1 M hydrochloric acid*, for preparing the *standard solutions* (25 ppm).

Sulphated ash (2.3.18). Not more than 5.0 per cent.

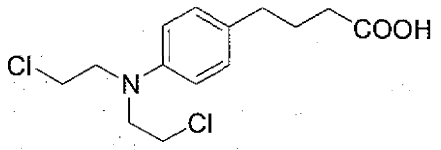
Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 120° for 4 hours.

Adsorbing power. Not less than 40 per cent of its own weight of *phenazone*, calculated on the dried basis, determined by the following method. To 0.3 g add 25 ml of a freshly prepared 1 per cent w/v solution of *phenazone*, shake thoroughly for 15 minutes, filter and discard the first 5 ml of the filtrate. To 10 ml of the filtrate add 1 g of *potassium bromide* and 20 ml of *2 M hydrochloric acid* and titrate with *0.0167 M potassium bromate*, using 0.1 ml of *methyl red solution* as indicator, until the colour changes from reddish pink to yellowish pink and titrate slowly towards the end of the titration (*a* ml). Repeat the titration using 10 ml of the phenazone solution beginning at the words "add 1 g..... titration" (*b* ml). Calculate the percentage of phenazone adsorbed with reference to the dried substance using the expression $2.353\ (a-b)/w$ where *w* is the weight, in g, of the substance under examination.

Microbial contamination (2.2.9). Total aerobic microbial count is not more than 10^3 CFU per g. Total fungal count is not more than 10^2 CFU per g. 1 g is free from *Escherichia coli* and 10 g is free from *Salmonella* and *Shigella*.

Storage. Store protected from moisture.

Chlorambucil



$C_{14}H_{19}Cl_2NO_2$ Mol. Wt. 304.2

Chlorambucil is 4-[4-bis(2-chloroethyl)amino]phenylbutyric acid.

Chlorambucil contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{14}H_{19}Cl_2NO_2$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A white, crystalline powder.

CAUTION — Chlorambucil must be handled with care; contact with the skin and inhalation of airborne particles must be avoided.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorambucil IPRS* or with the reference spectrum of chlorambucil.

B. Shake 0.4 g with 10 ml of *2 M hydrochloric acid* and allow to stand for 30 minutes, shaking occasionally. Filter, wash the residue with two quantities, each of 10 ml, of *water* and add 0.5 ml of *potassium mercuri-iodide solution* to 10 ml of the mixed filtrate and washings; a buff precipitate is produced. To a further 10 ml add 0.5 ml of *potassium permanganate solution*; the purple colour is immediately discharged.

C. Dissolve 50 mg in 5 ml of *acetone* and dilute to 10 ml with *water*. Add 0.05 ml of *2 M nitric acid* and 0.2 ml of *dilute silver nitrate solution*; no opalescence is produced immediately. Heat on a water-bath; an opalescence is produced.

Tests

Impurity G. Determine by liquid chromatography (2.4.14).

NOTE — The solutions are stable for 8 hours at room temperature or for 24 hours at 4° to 8° and protect them from light.

Test solution. Dissolve 10 mg of the substance under examination in *methanol* and dilute to 20.0 ml with *methanol*.

Reference solution. Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with phenylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of 1 per cent v/v solution of *trifluoroacetic acid,*
- flow rate: 1.8 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

The relative retention time with reference to chlorambucil (retention time is about 11 minutes) for 4-[2-[bis(2-chloroethyl)amino]phenyl]butanoic acid or 4-[3-[bis(2-chloroethyl)amino]phenyl]butanoic acid (chlorambucil impurity G) is about 1.2.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. The area of any peak due to chlorambucil impurity G is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.4 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use and protect from light.

Solvent mixture. 10 volumes of 1.0 per cent w/v solution of *hydrochloric acid* and 90 volumes of *acetonitrile.*

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 3.0 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.19 per cent w/v solution of *ammonium acetate*, adjusted to pH 3.9 with *acetic acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
5	60	40
15	10	90
25	10	90
26	60	40
30	60	40

Name	Relative retention time
Chlorambucil impurity B[1]	0.5
Chlorambucil (Retention time:about 12 minutes)	1.0
Chlorambucil impurity E[2]	1.4

[1]4-[4-[(2-chloroethyl)amino]phenyl]butanoic acid,

[2]4-[4-[[2-[[4-[4-[bis(2-chloroethyl)amino]phenyl]butanoyl]oxy]ethyl](2- chloroethyl)amino]phenyl]butanoic acid.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to chlorambucil impurity E is not more than 6 times the area of the principal peak in the chromatogram obtained with the reference solution (0.6 per cent), the area of any peak due to chlorambucil impurity B is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (0.4 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.2 g, dissolve in 10 ml of *acetone*, add 10 ml of *water* and titrate with *0.1 M sodium hydroxide* using *dilute phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03042 g of $C_{14}H_{19}Cl_2NO_2$.

Storage. Store protected from light.

Chlorambucil Tablets

Chlorambucil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorambucil, $C_{14}H_{19}Cl_2NO_2$. The tablets are coated.

Usual strengths. 2 mg; 5 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve one tablet as completely as possible in 10 ml of *0.1 M hydrochloric acid*, add 40 ml of *acetonitrile* and mix in an ultrasonic bath for 5 minutes. Add sufficient *acetonitrile* to produce a solution containing 0.002 per cent w/v of Chlorambucil. Filter the solution, preferably through a glass microfibre filter paper (such as Whatman GF/C), discarding the first 20 ml of the filtrate, and use the filtrate.

Reference solution. A 0.002 per cent w/v solution of *chlorambucil IPRS* in a mixture of 90 volumes of *acetonitrile* and 10 volumes of *0.1 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *0.02 M potassium dihydrogen phosphate*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Calculate the content of $C_{14}H_{19}Cl_2NO_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

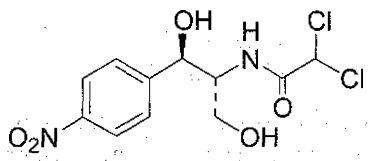
Test solution. Weigh and powder 20 tablets. Dissolve a quantity of the powder containing 10 mg of Chlorambucil in a mixture of 25 ml of *0.1 M hydrochloric acid* and 100 ml of *acetonitrile* by mixing in an ultrasonic bath for at least 10 minutes. Dilute to 250.0 ml with *acetonitrile* and filter the solution, preferably through a glass microfibre filter paper (such as Whatman GF/C), discarding the first 20 ml of the filtrate. Dilute 50.0 ml of the filtrate to 100.0 ml with a mixture of 90 volumes of *acetonitrile* and 10 volumes of *0.1 M hydrochloric acid.*

Reference solution. A 0.002 per cent w/v solution of *chlorambucil IPRS* in a mixture of 90 volumes of *acetonitrile* and 10 volumes of *0.1 M hydrochloric acid.*

Carry out the chromatographic procedure described under Uniformity of content.

Calculate the content of $C_{14}H_{19}Cl_2NO_2$ in the tablets.

Chloramphenicol



$C_{11}H_{12}Cl_2N_2O_5$ Mol. Wt. 323.1

Chloramphenicol is 2,2-dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-hydroxymethyl-2-(4-nitrophenyl)ethyl]acetamide. It is produced by the growth of certain strains of *Streptomyces venezuelae* in a suitable medium, but is normally prepared by synthesis.

Chloramphenicol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{11}H_{12}Cl_2N_2O_5$, calculated on the dried basis.

Category. Antibacterial.

Description. A white to greyish-white or yellowish-white, fine crystalline powder or fine crystals, needles or elongated plates.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chloramphenicol IPRS* or with the reference spectrum of chloramphenicol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 10 mg in 1 ml of *ethanol (50 per cent)*, add 3 ml of a 1 per cent w/v solution of *calcium chloride* and 50 mg of *zinc powder* and heat on a water-bath for 10 minutes. Decant the clear supernatant liquid into a test-tube, add 0.1 g of *anhydrous sodium acetate* and 0.1 ml of *benzoyl chloride*, shake for 1 minute and add 0.5 ml of a 10.5 per cent w/v solution of *ferric chloride hexahydrate* and, if necessary, add sufficient *dilute hydrochloric acid* to produce a clear solution; a red-violet to

purple colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

D. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 7.5, determined in a suspension prepared by shaking 50 mg with 10 ml of *carbon dioxide-free water.*

Specific optical rotation (2.4.22). +17.0° to +20.0°, determined in a 5.0 per cent w/v solution in *ethanol.*

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 90 volumes of *chloroform,* 10 volumes of *methanol* and 1 volume of *water.*

Test solution. Dissolve 1 g of the substance under examination in 100 ml of *acetone.*

Reference solution (a). A 1 per cent w/v solution of *chloramphenicol IPRS* in *acetone.*

Reference solution (b). Dilute 0.5 ml of reference solution (a) to 100 ml with *acetone.*

Apply to the plate 1 µl and 20 µl of the test solution, 1 µl of reference solution (a) and 20 µl of reference solution (b). After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with 20 µl of the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Chlorides (2.3.12). To 2.0 g add 20 ml of *water* and 10 ml of *nitric acid* and shake for 5 minutes. Filter through a filter paper previously washed by filtering 5-ml quantities of *water* until 5 ml of the filtrate is no longer opalescent on addition of 0.1 ml of *nitric acid* and 0.1 ml of a 4.25 per cent w/v solution of *silver nitrate.* The resulting filtrate complies with the limit test for chlorides (125 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.125 g and dissolve in sufficient *water* to produce 250.0 ml. Dilute 10.0 ml with sufficient *water* to produce 250.0 ml. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the specific absorbance at 278 nm.

Chloramphenicol intended for use in the manufacture of parenteral preparations without a further process for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of chloramphenicol.

Chloramphenicol intended for use in the manufacture of parenteral or ophthalmic preparations without a further sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture. If the material is intended for use in the manufacture of parenteral or ophthalmic preparations without a further appropriate procedure of sterilisation, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral or ophthalmic preparations.

Chloramphenicol Capsules

Chloramphenicol Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

Usual strengths. 250 mg; 500 mg.

Identification

Suspend a quantity of the contents of the capsules containing about 1.25 g of Chloramphenicol in 60 ml of *water* and extract with two quantities, each of 20 ml, of *light petroleum (60° to 80°)* or *light petroleum (100° to 120°).* Wash the combined extracts with two quantities, each of 15 ml, of *water,* add the washings to the aqueous layer, extract with four quantities, each of 50 ml, of *ether* and remove the ether from the combined extracts and evaporate to dryness. The residue, after drying to constant weight at 105°, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chloramphenicol IPRS* or with the reference spectrum of chloramphenicol.

B. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

Tests

Specific optical rotation (2.4.22). +17.0° to +20.0°, determined in a 5.0 per cent w/v solution in *ethanol* of the residue obtained in the test for Identification.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute 5.0 ml of the filtrate to 100.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the specific absorbance at 278 nm.

Q. Not less than 85 per cent of the stated amount of $C_{11}H_{12}Cl_2N_2O_5$.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.2 g of Chloramphenicol, dissolve in 800 ml of *water*, warming if necessary to effect solution and add sufficient *water* to produce 1000.0 ml. Dilute 10.0 ml of the solution to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$, taking 297 as the specific absorbance at 278 nm.

Storage. Store protected from moisture.

Chloramphenicol Ear Drops

Chloramphenicol Ear Drops are a solution of Chloramphenicol in a suitable vehicle.

Chloramphenicol Ear Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

Usual strengths. 0.4 per cent w/v; 0.5 per cent w/v.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica GF254.*

Mobile phase. A mixture of 1 volume of *water*, 10 volumes of *methanol* and 90 volumes of *chloroform.*

Test solution. Dilute a volume of the ear drops containing 0.1 g of Chloramphenicol to 10 ml with *ethanol (95 per cent).*

Reference solution. A 1.0 per cent w/v solution of *chloramphenicol IPRS* in *ethanol (95 per cent).*

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Dilute a volume of the ear drops containing 50 mg of Chloramphenicol to 10 ml with *ethanol (50 per cent).* To 2 ml, add 4.5 ml of *1 M sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10 M sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

Tests

2-Amino-1-(4-nitrophenyl)propane-1,3-diol. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the ear drops with the mobile phase to obtain a solution containing 0.05 per cent w/v of Chloramphenicol.

Reference solution (a). A 0.0025 per cent w/v solution of *2-amino-1-(4-nitrophenyl)propane-1,3-diol IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v, each of, *chloramphenicol IPRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol IPRS* in the mobile phase.

Use chromatographic system as described under Assay.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to chloramphenicol and 2-amino- 1-(4-nitrophenyl)propane-1,3-diol is not less than 8.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the solution, the area of any peak corresponding to 2-amino-1-(4-nitrophenyl)-propane-1,3-diol is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent).

Other tests. Comply with the tests stated under Ear Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the ear drops containing 25 mg of Chloramphenicol to 50 ml with *water.* Dilute 1.0 ml of the solution to 5.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *chloramphenicol IPRS* in *water.* Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v, each of, *chloramphenicol IPRS* and *2-amino-1-(4-nitrophenyl)propane-1,3-diol IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1 volume of *glacial acetic acid*, 15 volumes of *acetonitrile* and 85 volumes of 0.21 per cent w/v solution of *sodium pentanesulphonate*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to chloramphenicol and 2-amino- 1-(4-nitrophenyl)propane-1,3-diol is not less than 8.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the ear drops.

Storage. Store protected from light.

Chloramphenicol Eye Drops

Chloramphenicol Eye Drops are a sterile solution of Chloramphenicol in Purified water.

Chloramphenicol Eye Drops contain not less than 90.0 per cent and not more than 130.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

Usual strength. 0.5 per cent w/v.

Identification

To a volume containing 50 mg of Chloramphenicol add 15 ml of *water* and extract with four quantities, each of 25 ml, of *ether*. Combine the extracts and evaporate to dryness. The residue complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water*.

Test solution. Dissolve 0.1 g of the residue in sufficient *ethanol (95 per cent)* to produce 10 ml.

Reference solution. Dissolve 0.1 g of *chloramphenicol IPRS* in sufficient *ethanol (95 per cent)* to produce 10 ml.

Apply to the plate 1µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Dissolve 10 mg in 2 ml of *ethanol (50 per cent)*, add 4.5 ml of *1 M sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter, if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10 M sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

Tests

pH (2.4.24). 7.0 to 7.5.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the eye drops containing about 50 mg of chloramphenicol to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 25.0 ml with the mobile phase and filter through a 0.5 µm or finer porosity filter and use the clear filtrate.

Reference solution. A 0.01 per cent w/v solution of *chloramphenicol IPRS* in the mobile phase. Filter the solution through a 0.5 µm or finer porosity filter and use the clear filtrate.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *water*, 45 volumes of *methanol* and 0.1 volume of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the eye drops.

Storage. Store in light resistant containers at a temperature not exceeding 30°.

Chloramphenicol Eye Ointment

Chloramphenicol Eye Ointment contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

Usual strength. 1 per cent w/w.

Identification

Mix a quantity of the eye ointment containing 30 mg of Chloramphenicol with 10 ml of *light petroleum (40° to 60°)*,

centrifuge and discard the supernatant liquid. Repeat this procedure using three quantities, each of 10 ml, of the same solvent. Dry the residue in oven at 105°. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chloramphenicol IPRS* or with the reference spectrum of chloramphenicol.

B. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

Tests

Other tests. Comply with the tests stated under Eye Ointments.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately weighed quantity of the ointment, containing about 25 mg of Chloramphenicol, to a suitable conical flask, add 20 ml of *cyclohexane*, mix with the aid of ultrasound for about 2 minutes add 60 ml of *methanol*, and mix. Filter this mixture, collecting the filtrate in a 100-ml volumetric flask. Wash the filter with *methanol*, collecting the washings in the volumetric flask. Dilute with *methanol* to volume, and mix. Transfer 50.0 ml of the resulting solution to a suitable round-bottom flask, and evaporate to dryness by rotating the flask under vacuum in a water-bath at 35°. Dissolve the residue in 50.0 ml of *methanol*. Transfer 10.0 ml of the resulting solution to a 25-ml volumetric flask, dilute with the mobile phase to volume, and mix. Filter a portion of the solution through a 0.5 µm or finer porosity filter, and use the clear filtrate.

Reference solution. A 0.01 per cent w/v solution of *chloramphenicol IPRS* in the mobile phase. Filter the solution through a 0.5 µm or finer porosity filter and use the clear filtrate.

Chromatographic system

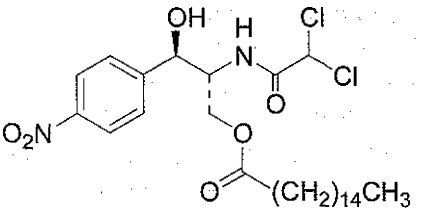
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *water*, 45 volumes of *methanol* and 0.1 volume of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the eye ointment.

Storage. Store at a temperature not exceeding 30°.

Chloramphenicol Palmitate



$C_{27}H_{42}Cl_2N_2O_6$ Mol. Wt. 561.6

Chloramphenicol Palmitate is (2*R*,3*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-3-(4-nitrophenyl)propyl hexadecanoate.

Chloramphenicol Palmitate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{27}H_{42}Cl_2N_2O_6$, calculated on the dried basis.

Category. Antibacterial.

Description. A fine, white or almost white, unctuous powder.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 271 nm, about 0.53.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel H*.

Mobile phase. A mixture of 70 volumes of *ethanol (95 per cent)* and 30 volumes of 10 per cent w/v solution of *ammonium acetate*.

Test solution. Dissolve 50 mg of the substance under examination in a mixture of 1 ml of *1 M sodium hydroxide* and 5 ml of *acetone*, allow to stand for 30 minutes and add 1.1 ml of *1 M hydrochloric acid* and 3 ml of *acetone*.

Reference solution (a). A 0.2 per cent w/v solution of *chloramphenicol IPRS* in *acetone*.

Reference solution (b). A 0.2 per cent w/v solution of *palmitic acid* in *acetone*.

Reference solution (c). A 0.2 per cent w/v solution of the substance under examination in *acetone*.

Apply to the plate 4 µl of each solution. After development, dry the plate in air and spray with a solution containing 0.02 per cent w/v of *2,7-dichlorofluorescein* and 0.01 per cent w/v of *rhodamine B* in *ethanol (95 per cent)*. Allow the plate to dry in air and examine under ultraviolet light at 254 nm. The chromatogram obtained with the test solution shows three spots corresponding in position to the principal spots in chromatograms obtained with reference solution (a), (b) and (c).

C. Dissolve 10 mg in 4 ml of *ethanol (95 per cent)* add 1 ml of *1 M sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Filter, cool the filtrate in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10 M sodium hydroxide*; a red colour develops. Repeat the test omitting the zinc powder; no red colour is produced.

D. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* in a covered test-tube on a water-bath for 15 minutes; the resulting solution gives the reactions of chlorides (2.3.1).

Tests

Specific optical rotation (2.4.22). +21.0° to +25.0°, determined in a 5.0 per cent w/v solution in *ethanol*.

Free acid. Dissolve 1.0 g by warming to 35° in 5 ml of a mixture of a equal volumes of *ethanol (95 per cent)* and *ether* and add 0.2 ml of *phenolphthalein solution*; not more than 0.4 ml of *0.1 M sodium hydroxide* is required to produce a pink colour persisting for 30 seconds.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Dissolve 1 g of the substance under examination in 100.0 ml of *acetone*.

Reference solution. Dilute 2.0 ml of the test solution to 100.0 ml with *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Free chloramphenicol. Not more than 450 ppm, determined by the following method. Dissolve, with the aid of gentle heat, 1.0 g in 80 ml of *xylene*, cool and extract with three successive quantities, each of 15 ml, of *water*; discard the xylene and dilute the combined aqueous extracts to 50 ml with *water*. Extract the solution with 10 ml of *carbon tetrachloride*, allow to separate, discard the carbon tetrachloride and centrifuge a portion of the aqueous solution. Measure the absorbance of the clear aqueous solution at the maximum at about 278 nm, using as the blank a solution obtained by repeating the procedure without the substance under examination; the absorbance of this blank solution must not be greater than 0.05 (2.4.7). Calculate the content of free chloramphenicol, in ppm, from the expression $(A \times 10^4)/5.96$, where A is the absorbance of the clear aqueous solution of the substance under examination.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 80° at a pressure not exceeding 0.1 kPa for 3 hours.

Assay. Weigh accurately about 60 mg and dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml. Dilute 10.0 ml of the solution to 200.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_{27}H_{42}Cl_2N_2O_6$ taking 178 as the specific absorbance at 271 nm.

Storage. Store protected from light and moisture.

Chloramphenicol Oral Suspension

Chloramphenicol Palmitate Oral Suspension; Chloramphenicol Palmitate Mixture

Chloramphenicol Oral Suspension is a suspension of Chloramphenicol Palmitate in a suitable flavoured vehicle.

Chloramphenicol Oral Suspension contains not less than 95.0 per cent and not more than 115.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

Usual strength. The equivalent of 125 mg of chloramphenicol per 5 ml. (175 mg of chloramphenicol palmitate is approximately equivalent to 100 mg of chloramphenicol).

Identification

Extract a quantity of the suspension containing about 7.5 mg of chloramphenicol with 10 ml of *chloroform* and carefully evaporate the clear chloroform solution on a water-bath to dryness. Dissolve the residue in 250 ml of *ethanol (95 per cent.* When examined in the range 230 nm to 360 nm (2.4.7) the resulting solution shows an absorption maximum only at about 271 nm.

Tests

pH (2.4.24). 4.5 to 7.0.

Polymorph A. To a volume of the suspension containing 0.125 g of chloramphenicol add 35 ml of *water*, mix, centrifuge for 40 minutes at not less than 18,000 rpm and discard the supernatant liquid. Wash the residue by adding 2 ml of *water*, triturating to form a paste, adding 18 ml of *water*, mixing thoroughly centrifuging and discarding the supernatant liquid. Wash the residue twice more in a similar manner, dry at 20° for 16 hours at a pressure not exceeding 0.7 kPa and grind to a fine powder. Prepare a mull of the residue by triturating a small quantity with about twice its weight of *liquid paraffin* until a smooth creamy paste is obtained. Determine by infrared absorption spectrophotometry (2.4.6) over the range 770 cm^{-1} to 910 cm^{-1} using conditions such that between 20 per cent and 30 per cent transmittance occurs at 810 cm^{-1} to 910 cm^{-1}.

Repeat the operation using a mull prepared with a standard mixture obtained by mixing together thoroughly 1 part by weight of *chloramphenicol palmitate polymorph A IPRS* and 9 parts by weight of *chloramphenicol palmitate nonpolymorph A IPRS*. On each of the spectra, draw a straight base line between the minima occurring at about 880 cm^{-1} and 790 cm^{-1} and using these base lines measure the heights of the peaks occuring at the maxima at about 858 cm^{-1} and 840 cm^{-1}. In the spectrum obtained with preparation under examination, the ratio of the peak height at about 858 cm^{-1} to that at the maximum at about 840 cm^{-1} is greater than the corresponding ratio in the spectrum obtained with the standard mixture.

Other tests. Comply with the tests stated under Oral Liquids.

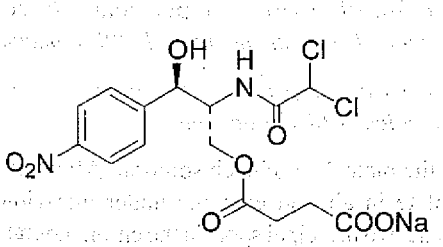
Assay. Weigh accurately a quantity of the suspension containing about 0.125 g of chloramphenicol, add 10 ml of *water* and shake with four quantities, each of 20 ml, of *chloroform*, filtering each extract through cotton wool, previously washed with *chloroform*, into a 100-ml volumetric flask. Dilute to volume with *chloroform* and mix well. Dilute 2.0 ml of the solution to 100.0 with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 271 nm using 1 ml of *chloroform* diluted to 50 ml with *ethanol (95 per cent)* as the blank (2.4.7). Calculate the content of chloramphenicol palmitate, $C_{27}H_{42}Cl_2N_2O_6$, taking 178 as the specific absorbance at 271 nm.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$, weight in volume using a factor of 0.575 for the conversion of the content of chloramphenicol palmitate to chloramphenicol.

Storage. Store protected from light.

Labelling. The label states (1) the strength in terms of the equivalent amount of chloramphenicol; (2) that if the preparation is diluted, it must be used immediately after dilution.

Chloramphenicol Sodium Succinate



$C_{15}H_{15}Cl_2N_2NaO_8$ Mol. Wt. 445.2

Chloramphenicol Sodium Succinate is a mixture of variable proportions of sodium (2*R*,3*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-3-(4-nitrophenyl)propyl succinate (3-isomer) and of sodium (1*R*,2*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-1-(4-nitrophenyl)propyl succinate (1-isomer).

Chloramphenicol Sodium Succinate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{15}Cl_2N_2NaO_8$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or yellowish-white powder; hygroscopic.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of *2 M acetic acid*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *acetone*.

Reference solution (a). A 1 per cent w/v solution of *chloramphenicol sodium succinate IPRS* in *acetone*.

Reference solution (b). A 1 per cent w/v solution of *chloramphenicol IPRS* in *acetone*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution are similar in position and size to those in the chromatogram obtained with reference solution (a) and their positions are different from that of the principal spot in the chromatogram obtained with reference solution (b).

B. Dissolve 10 mg in 2 ml of *ethanol (95 per cent)* add 4.5 ml of *1 M sulphuric acid* and 50 mg of *zinc powder*, allow to stand for 10 minutes and decant the supernatant liquid or filter, if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10 M sodium hydroxide*; a red colour develops. Repeat the test omitting the zinc powder; no red colour is produced.

C. To 5 ml of a 0.1 per cent w/v solution add a few drops of *silver nitrate solution*; no precipitate is produced. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* on a water-bath for 15 minutes, add 50 mg of *decolorising charcoal*, shake and filter. The filtrate when treated with *silver nitrate solution*, yields a curdy precipitate which is insoluble in *nitric acid* but soluble, after being well washed with *water*, in *dilute ammonia solution* from which it is reprecipitated on addition of *nitric acid*.

D. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 6.4 to 7.0, determined in a 25.0 per cent w/v solution.

Specific optical rotation (2.4.22). + 5.0° to + 8.0°, determined in a 5.0 per cent w/v solution.

Free chloramphenicol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *acetone*.

Reference solution. A 0.02 per cent w/v solution of *chloramphenicol IPRS* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any spot corresponding to chloramphenicol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

Assay. Weigh accurately about 0.2 g and dissolve in sufficient *water* to produce 500.0 ml; dilute 5.0 ml of the solution to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 276 nm (2.4.7). Calculate the content of $C_{15}H_{15}Cl_2N_2NaO_8$ taking 220 as the specific absorbance at 276 nm.

Chloramphenicol Sodium Succinate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of chloramphenicol.

Chloramphenicol Sodium Succinate intended for use in the manufacture of parenteral preparations without a further sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture. If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Chloramphenicol Sodium Succinate Injection

Chloramphenicol Sodium Succinate Injection is a sterile material consisting of Chloramphenicol Sodium Succinate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Chloramphenicol Sodium Succinate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

Usual strengths. The equivalent of 300 mg and 1 g of chloramphenicol. (140 mg of chloramphenicol sodium succinate is approximately equivalent to 100 mg of chloramphenicol).

Description. A white or yellowish-white powder; hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powder for Injections) and with the following requirements.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of *2 M acetic acid*.

Test solution. Dissolve a quantity of injection containing 0.1 g of chloramphenicol sodium succinate in *acetone* and dilute to 10.0 ml with *acetone*.

Reference Solution (a). A 1 per cent w/v solution of *chloramphenicol sodium succinate IPRS* in *acetone*.

Reference solution (b). A 1 per cent w/v solution of *chloramphenicol IPRS* in *acetone*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution are similar in position and size to those in the chromatogram obtained with reference solution (a) and their positions are different from that of the principal spot in the chromatogram obtained with reference solution (b).

B. Dissolve 10 mg in 2 ml of *ethanol (95 per cent)* add 4.5 ml of *1 M sulphuric acid* and 50 mg of *zinc powder*, allow to stand for 10 minutes and decant the supernatant liquid or filter, if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10 M sodium hydroxide*; a red colour develops. Repeat the test omitting the zinc powder; no red colour is produced.

C. To 5 ml of a 0.1 per cent w/v solution add a few drops of *silver nitrate solution*; no precipitate is produced. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* on a water-bath for 15 minutes, add 50 mg of *decolorising charcoal*, shake and filter. The filtrate when treated with *silver nitrate solution*, yields a curdy precipitate which is insoluble in *nitric acid* but soluble, after being well washed with *water*, in *dilute ammonia solution* from which it is reprecipitated on addition of *nitric acid*.

D. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 6.4 to 7.0, determined in a 25.0 per cent w/v solution.

Specific optical rotation (2.4.22). + 5.0° to + 8.0°, determined in a 5.0 per cent w/v solution.

Free chloramphenicol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water*.

Test solution. Dissolve a quantity of injection containing 0.1 g of chloramphenicol sodium succinate in *acetone* and dilute to 10.0 ml with *acetone*.

Reference solution. A 0.02 per cent w/v solution of *chloramphenicol IPRS* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any spot corresponding to chloramphenicol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of chloramphenicol.

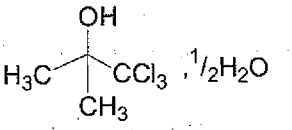
Assay. Determine the weight of the contents of 10 containers. Weigh accurately about 0.2 g of the mixed contents of the 10 containers and dissolve in sufficient *water* to produce 500.0 ml; dilute 5.0 ml of the solution to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 276 nm (2.4.7). Calculate the content of $C_{15}H_{15}Cl_2N_2NaO_8$ taking 220 as the specific absorbance at 276 nm. 1 mg of $C_{15}H_{15}Cl_2N_2NaO_8$ is equivalent to 0.7257 mg of $C_{11}H_{12}Cl_2N_2O_5$.

Storage. Store protected from light and moisture.

Labelling. The label states the quantity of Chloramphenicol Sodium Succinate in the sealed container in terms of the equivalent amount of chloramphenicol.

Chlorbutol

Chlorobutanol



$C_4H_7Cl_3O, ½ H_2O$ Mol.Wt. 186.5

Chlorbutol is 1,1,1-trichloro-2-methylpropan-2-ol hemihydrate.

Chlorbutol contains not less than 98.0 per cent and not more than 101.0 per cent of $C_4H_7Cl_3O$, calculated on the anhydrous basis.

Category. Pharmaceutical aid (antimicrobial preservative), analgesic; local anaesthetic.

Description. Colourless crystals or a white, crystalline powder; sublimes readily.

Identification

A. To 5 ml of a freshly prepared 0.5 per cent w/v solution add 1 ml of *1 M sodium hydroxide* and then, slowly, 2 ml of *iodine solution*; a yellow precipitate of iodoform is produced.

B. Heat about 20 mg with 2 ml of *10 M sodium hydroxide* and 1 ml of *pyridine* on a water-bath and shake; the separated pyridine layer becomes red.

C. Warm gently about 20 mg with 5 ml of *ammoniacal silver nitrate solution*; a black precipitate is produced.

Tests

Appearance of solution. A 50.0 per cent w/v solution in *ethanol (95 per cent)* is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

Acidity. Dissolve 2.0 g in 20 ml of *ethanol (95 per cent)*, add 0.1 ml of *bromothymol blue solution* and titrate with *0.1 M*

sodium hydroxide; not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Chlorides (2.3.12). 0.5 g dissolved in 10 ml of *ethanol (95 per cent)* complies with the limit test for chlorides (500 ppm). Use 5 ml of *ethanol (95 per cent)* in place of 5 ml of *water* to prepare the standard.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

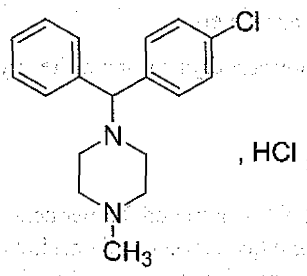
Water (2.3.43). 4.5 per cent to 6.0 per cent, determined on 0.3 g.

Assay. Weigh accurately about 0.2 g and dissolve in 5 ml of *ethanol (95 per cent)*. Add 5 ml of *sodium hydroxide solution* and boil under a reflux condenser for 15 minutes. Cool, dilute with 20 ml of *water*, add 5 ml of *nitric acid*, 1 ml of *nitrobenzene* and 50.0 ml of *0.1 M silver nitrate* and shake vigorously for 1 minute. Add 4 ml of *ferric ammonium sulphate solution* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate*.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005917 g of $C_4H_7Cl_3O$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Chlorcyclizine Hydrochloride



$C_{18}H_{21}ClN_2,HCl$ Mol. Wt. 337.3

Chlorcyclizine Hydrochloride is 1-(4-chlorobenzhydryl)-4-methylpiperazine hydrochloride.

Chlorcyclizine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of the stated amount of $C_{18}H_{21}ClN_2,HCl$, calculated on the dried basis.

Category. Antihistaminic.

Description. A white crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorcyclizine hydrochloride IPRS* or with the reference spectrum of chlorcyclizine hydrochloride.

B. Weigh accurately about 10 mg, dissolve in 100 ml of 0.5 per cent w/v of *sulphuric acid*. Dilute 10 ml of the solution to 100 ml with 0.5 per cent w/v *sulphuric acid*. When examined in the range 215 to 300 nm (2.4.7), exhibits maximum at about 231 nm, about 0.475 to 0.525.

C. In the test for Related substances, the principle spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. It gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 5.0 to 6.0, determined in a 1.0 per cent w/v solution.

Related substance. Determine by thin layer chromatography (2.4.17). coating the plate with *silica gel*.

Mobile phase. A mixture of 85 volumes of *dichloromethane*, 13 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

Test solution (a). A 2.0 per cent w/v solution of the substance under examination in *methanol*.

Test solution (b). A 0.1 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (a). A 0.10 per cent w/v solution of *chlorcyclizine hydrochloride IPRS* in *methanol*.

Reference solution (b). A 0.01 per cent w/v solution of *methylpiperzine IPRS* in *methanol*.

Reference solution (c). A 0.004 per cent w/v solution of the substance under examination in *methane*.

Reference solution (d). A solution containing 0.10 per cent w/v, each of, *hydroxyzine hydrochloride IPRS* and *chlorcyclizine hydrochloride IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapours for 10 minutes. In the chromatogram obtained with test solution (a), any spot corresponding to methylpiperazine is not more intense than the spot in the chromatogram obtained with reference solution (b)(0.5 per cent). Any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

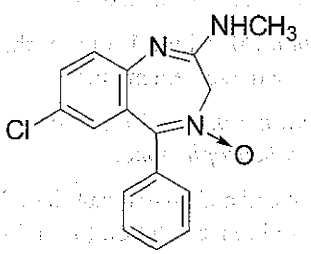
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 130°.

Assay. Weigh accurately about 0.2 g, dissolve in 1 ml of *0.1 M hydrochloric acid* and add 50 ml of *methanol*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03373 g of $C_{18}H_{21}ClN_2HCl$.

Storage. Store protected from light and moisture.

Chlordiazepoxide



$C_{16}H_{14}ClN_3O$ Mol. Wt. 299.8

Chlordiazepoxide is 7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide.

Chlordiazepoxide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{14}ClN_3O$, calculated on the dried basis.

Category. Anxiolytic.

Description. An almost white to light yellow, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlordiazepoxide IPRS* or with the reference spectrum of chlordiazepoxide.

B. When examined in the range 230 nm to 360 nm (2.4.7) a 0.0005 per cent w/v solution prepared immediately before use in subdued light in *0.1 M hydrochloric acid* shows absorption maxima at about 246 nm and 308 nm. Absorbance at the maximum at about 246 nm, 0.56 to 0.60 and at the maximum at about 308 nm, 0.16 to 0.17.

C. Dissolve 0.2 g in 4 ml of hot *dilute hydrochloric acid*, heat at 100° for 10 minutes, cool and filter. 2 ml of the filtrate gives the reactions of primary aromatic amines (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions and protected from light.

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *chlordiazepoxide impurity A IPRS* in the mobile phase, add 25.0 ml of the test solution and dilute to 100.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (c). Dissolve 4 mg of *aminochlorobenzophenone* in 100 ml of the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

The relative retention time with reference to chlordiazepoxide for 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide (chlordiazepoxide impurity A) is about 0.7; for 6-chloro-2-(chloromethyl)-4-phenylquinazoline 3-oxide (chlordiazepoxide impurity B) is about 2.3; for aminochlorobenzophenone (chlordiazepoxide impurity C) is about 3.9.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to chlordiazepoxide impurity A and chlordiazepoxide is not less than 5.0.

Inject reference solution (a), (c) and the test solution. Run the chromatogram 6 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to chlordiazepoxide impurities A and B, is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any peak corresponding to chlordiazepoxide impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram

obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g and dissolve by heating, if necessary, in 80 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02998 g of $C_{16}H_{14}ClN_3O$.

Storage. Store protected from light and moisture.

Chlordiazepoxide Tablets

Chlordiazepoxide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlordiazepoxide, $C_{16}H_{14}ClN_3O$.

Usual strengths. 5 mg; 10 mg; 25 mg.

Identification

A. Dilute 1 ml of the final solution obtained in the Assay to 2 ml with *0.1 M hydrochloric acid*. When examined in the range 230 nm to 360 nm (2.4.7) the resulting solution shows absorption maxima at about 246 nm and 308 nm.

B. To a quantity of the powdered tablets containing 0.2 g of Chlordiazepoxide add 4 ml of hot *2 M hydrochloric acid*, heat at 100° for 10 minutes, cool and filter; 2 ml of the filtrate gives the reactions of primary aromatic amines (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium: 900 ml of gastric juice, artificial (without enzyme),
Speed and time. 100 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium, if necessary. Measure the absorbance of the resulting solution at the maximum at about 309 nm (2.4.7). Calculate the content of chlordiazepoxide, $C_{16}H_{14}ClN_3O$ in the medium from the absorbance obtained from a solution of known concentration of *chlordiazepoxide IPRS* in the dissolution medium.

Q. Not less than 85 per cent of the stated amount of $C_{16}H_{14}ClN_3O$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Chlordiazepoxide with 10 ml of a mixture of *acetone* containing 2 per cent v/v of *strong ammonia solution* and 8 per cent v/v of *water*, allow to settle and use the clear supernatant liquid.

Reference solution (a). Dilute 5.0 ml of the test solution to 100.0 ml with the same solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the same solvent mixture.

Reference solution (c). A 0.01 per cent w/v solution of *2-amino-5-chlorobenzophenone*.

Apply to the plate 2 µl and 20 µl quantities of the test solution, 2 µl of each of reference solution (a) and (b) and 20 µl of reference solution (c). After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with 2 µl of the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a freshly prepared 1 per cent w/v solution of *sodium nitrite* in *1 M hydrochloric acid*, dry it in a current of air and spray with a 0.4 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)*. Any violet spot corresponding to 2-amino-5-chlorobenzophenone in the chromatogram obtained with 20 µl of the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

Uniformity of content. Complies with the test stated under Tablets.

Powder one tablet, shake with 50 ml of *0.1 M hydrochloric acid* for 20 minutes and add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml. Filter and dilute a suitable volume of the filtrate containing 0.8 mg of Chlordiazepoxide with sufficient *0.1 M hydrochloric acid* to produce 50.0 ml. Measure the absorbance of the resulting solution at the maximum at about 308 nm (2.4.7). Calculate the content of $C_{16}H_{14}ClN_3O$ in the tablet taking 327 as the specific absorbance at 308 nm.

Other tests. Comply with the tests stated under Tablets.

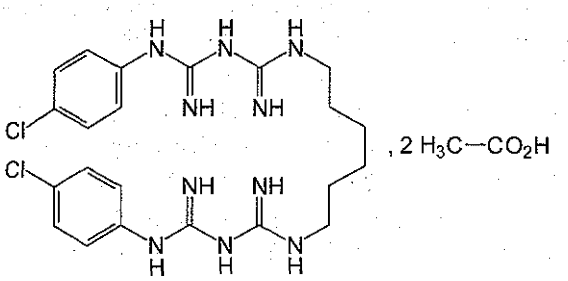
Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 20 mg of Chlordiazepoxide and

shake with 150 ml of *0.1 M hydrochloric acid* for 20 minutes. Add sufficient *0.1 M hydrochloric acid* to produce 250.0 ml and filter. Dilute 10.0 ml of the filtrate to 50.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 308 nm (2.4.7). Calculate the content of $C_{16}H_{14}ClN_3O$ taking 327 as the specific absorbance at 308 nm.

Storage. Store protected from light at a temperature not exceeding 30°.

Chlorhexidine Acetate

Chlorhexidine Diacetate



$C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$ Mol. Wt. 625.6

Chlorhexidine Acetate is 1,1'-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] diacetate.

Chlorhexidine Acetate contains not less than 98.0 per cent and not more than 101.0 per cent of chlorhexidine diacetate, $C_{22}H_{30}Cl_2N_{10},2C_2H_4O_2$, calculated on the dried basis.

Category. Antiseptic.

Description. A white or almost white, microcrystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorhexidine acetate IPRS* or with the reference spectrum of chlorhexidine acetate.

B. Dissolve about 5 mg in 5 ml of a warm 1.0 per cent w/v solution of *cetrimide* and add 1 ml of *strong sodium hydroxide solution* and 1 ml of *bromine water*. A deep red colour is produced.

C. Dissolve 0.3 g in 10 ml of a mixture of equal volumes of *hydrochloric acid* and *water*. Add 40 ml of *water*, filter if necessary and cool in ice *water*. Make alkaline to *titan yellow paper* by adding dropwise and with stirring *strong sodium hydroxide solution* and add 1 ml in excess. Filter, wash the precipitate with *water* until the washings are free from alkali and recrystallise from *alcohol (70 per cent v/v)*. Dry at 100° to 105°. Melting point (2.4.21). 132° to 136°.

D. It gives reaction (A) of acetates (2.3.1).

Tests

Chloroaniline. Dissolve 0.2 g of the substance under examination in 25 ml of *water* with shaking if necessary. Add 1 ml of *hydrochloric acid* and dilute to 30 ml with *water*. Add rapidly and with thorough mixing after each addition, 2.5 ml of *dilute hydrochloric acid*, 0.35 ml of *sodium nitrite solution*, 2 ml of a 5.0 per cent w/v solution of *ammonium sulphamate*, 5 ml of a 0.1 per cent w/v solution of *naphthylethylenediamine dihydrochloride* and 1 ml of *alcohol*, dilute to 50.0 ml with *water* and allow to stand for 30 minutes. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 10.0 ml of 0.001 per cent w/v solution of *chloroaniline* in *dilute hydrochloric acid* and 20 ml of *dilute hydrochloric acid* instead of the solution of the substance under examination (500 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.15 per cent w/v solution of *chlorhexidine acetate IPRS* in the mobile phase.

Reference solution (b). Dilute 2.5 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c) Dilute 2.0 ml of reference solution (b) to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 2.0 g of *sodium octanesulphonate* in a mixture of 120 ml of *glacial acetic acid*, 270 ml of *water* and 730 ml of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Equilibrate the column with the mobile phase for at least 1 hour. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 50 per cent of the full scale of the recorder.

Inject the test solution and reference solution (a), (b) and (c). Record the chromatograms of reference solution (b) and (c)

until the peak due to chlorhexidine has been eluted and record the chromatogram of the test solution for six times the retention time of the peak due to chlorhexidine. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks, other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with a relative retention time of 0.25 or less with respect to the principal peak and any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (c).

Loss on drying (2.4.19). Not more than 3.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Sulphated ash (2.3.18). Not more than 0.15 per cent.

Assay. Dissolve 0.14 g in 100 ml of *anhydrous acetic acid* and titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M perchloric acid* is equivalent to 0.01564 g of $C_{26}H_{38}Cl_2N_{10}O_4$.

Chlorhexidine Gluconate Solution

Chlorhexidine Gluconate Solution is an aqueous solution of 1,1'-hexamethylenebis [5-(4-chlorophenyl)biguanide] digluconate.

Chlorhexidine Gluconate Solution contains not less than 19.0 per cent w/v and not more than 21.0 per cent w/v of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

Category. Antiseptic.

Description. An almost colourless or pale yellowish, clear or slightly opalescent liquid.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out

A. To 2 ml add 80 ml of *water*, cool in ice, add *5 M sodium hydroxide* dropwise with stirring until the solution is slightly alkaline to titan yellow paper and add 2 ml in excess. Filter, wash the precipitate with *water* until the washings are free from alkali, dissolve it in about 25 ml of *ethanol* on a boiling water-bath and heat until the volume is reduced to about 5 ml. Cool in ice, induce crystallisation, if necessary, by scratching the side of the vessel with a glass rod, filter and dry the crystals at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorhexidine IPRS* or with the reference spectrum of chlorhexidine. Examine the substance as a dispersion in *potassium bromide IR* without excessive grinding.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *ethanol (95 per cent)*, 30 volumes of *water*, 10 volumes of *strong ammonia solution* and 10 volumes of *ethyl acetate*.

Test solution. Dilute 10 ml of the substance under examination to 50.0 ml with *water*.

Reference solution. A 2.5 per cent w/v solution of *calcium gluconate IPRS* in *water*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 100° for 20 minutes, allow to cool, spray with a 5 per cent w/v solution of *potassium dichromate* in a 40 per cent w/w solution of *sulphuric acid* and allow to stand for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. To 0.5 ml add 10 ml of *water* and 0.5 ml of *cupric sulphate solution*; a white precipitate is produced which on boiling flocculates and changes to a pale purple colour.

D. To 0.05 ml add 5 ml of a 1 per cent w/v solution of *cetrimide*, 1 ml of *10 M sodium hydroxide* and 1 ml of *bromine water*; a deep red colour is produced.

Tests

pH (2.4.24). 5.5 to 7.0, determined in a solution obtained by diluting 5 ml to 100 ml.

Weight per ml (2.4.29). 1.06 g to 1.07 g.

Related substances. Determine by thin-layer chromatography (2.4.17), coating a 0.5-mm thick plate with a slurry consisting of 8 g of *silica gel GF254* and 16 ml of *water* containing 1 g of *sodium formate*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 50 volumes of *ethanol (95 per cent)* and 7 volumes of *formic acid*.

Test solution. Dilute 1 ml of the substance under examination to 20 ml with *1.5 M acetic acid*.

Apply to the plate, in the form of a band 4 cm wide, 20 µl of the test solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Mark the area around each group of bands above and below the principal band, transfer quantitatively the enclosed areas of silica gel to a glass-stoppered tube, add 5.0 ml of *methanol*, shake for 15 minutes, centrifuge and measure the absorbance of the clear, supernatant liquid at the maximum at about 256 nm (2.4.7), using as the blank a solution prepared by heating in a similar manner equivalent-sized areas of silica gel removed from the coating adjacent to the areas previously removed. The absorbance is not more than that obtained with a solution prepared by

diluting 2 ml of the substance under examination with sufficient *1.5 M acetic acid* to produce 10 ml and diluting 0.2 ml of the solution to 50 ml with *methanol*.

4-Chloroaniline. Not more than 0.25 per cent, calculated with reference to chlorhexidine solution at a nominal concentration of 20 per cent w/v, determined by the following method. Dilute 2.0 ml to 100.0 with *water*. To 10.0 ml of the solution add 2.5 ml of *2 M hydrochloric acid* and dilute to 20 ml with *water*. Add rapidly, with continuous mixing after each addition, 0.35 ml of *sodium nitrite* solution, 2 ml of a 5 per cent w/v solution of *ammonium sulphamate* and 5 ml of a 0.01 per cent w/v solution of *N-(1-naphthyl) ethylenediamine dihydrochloride*. Add 1 ml of *ethanol (95 per cent)* and sufficient *water* to produce 50 ml, mix and set aside for 30 minutes. Any reddish blue colour produced is not more intense than that produced by treating at the same time in the same manner a mixture of 10.0 ml of 0.001 per cent w/v solution of *4-chloroaniline* in *2 M hydrochloric acid* and 10 ml of *water* in place of the dilution of the substance under examination.

Assay. Weigh accurately about 1.0 g and evaporate to a low bulk. Dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

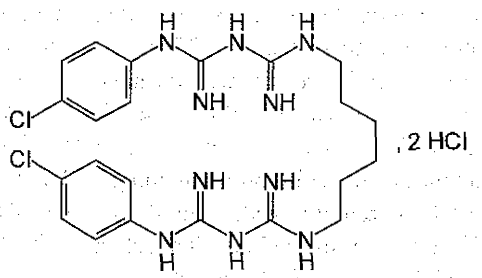
1 ml of *0.1 M perchloric acid* is equivalent to 0.02244 g of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

Determine the weight per ml (2.4.29) and calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$, weight in volume.

Storage. Store protected from light.

Chlorhexidine Hydrochloride

Chlorhexidine Dihydrochloride



$C_{22}H_{30}Cl_2N_{10},2HCl$ Mol. Wt. 578.4

Chlorhexidine Hydrochloride is 1,1'-(hexane-1,6-diyl)bis[5-(4-chlorophenyl)biguanide] dihydrochloride.

Chlorhexidine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of chlorhexidine dihydrochloride, $C_{22}H_{30}Cl_2N_{10}$, 2HCl calculated on the dried basis.

Category. Antiseptic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorhexidine hydrochloride IPRS* or with the reference spectrum of chlorhexidine hydrochloride.

B. Dissolve about 5 mg in 5 ml of a warm 1.0 per cent w/v solution of *cetrimide* and add 1 ml of *strong sodium hydroxide solution* and 1 ml of *bromine water*. A deep red colour is produced.

C. Dissolve 0.3 g in 10 ml of a mixture of equal volumes of *hydrochloric acid* and *water*. Add 40 ml of *water*, filter if necessary and cool in ice *water*. Make alkaline to titan yellow paper by adding dropwise and with stirring *strong sodium hydroxide solution* and add 1 ml in excess. Filter, wash the precipitate with *water* until the washings are free from alkali and recrystallise from *alcohol (70 per cent v/v)*. Dry at 100° to 105°. Melting point (2.4.21). 132° to 136°.

D. It gives reaction (a) of chlorides (2.3.1).

Tests

Chloroaniline. To 0.2 g of the substance under examination, add 1 ml of *hydrochloric acid*, dilute to 30 ml with *water* and shake until a clear solution is obtained. Add rapidly and with thorough mixing after each addition, 2.5 ml of *dilute hydrochloric acid*, 0.35 ml of *sodium nitrite solution*, 2 ml of a 5.0 per cent w/v solution of *ammonium sulphamate*, 5 ml of a 0.1 per cent w/v solution of *naphthylethylenediamine dihydrochloride* and 1 ml of *alcohol*, dilute to 50.0 ml with *water* and allow to stand for 30 minutes. Any reddish-blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 10.0 ml of a 0.001 per cent solution of *chloroaniline* in *dilute hydrochloric acid* and 20 ml of *dilute hydrochloric acid* instead of the solution of the substance under examination (500 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.15 per cent w/v solution of *chlorhexidine hydrochloride IPRS* in the mobile phase.

Reference solution (b). Dilute 2.5 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 2.0 ml of reference solution (b) to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 2.0 g of *sodium octanesulphonate* in a mixture of 120 ml of *glacial acetic acid*, 270 ml of *water* and 730 ml of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Equilibrate the column with the mobile phase for at least 1 hour.

Inject reference solution (a), (b), (c) and the test solution. Record the chromatograms until the peak due to chlorhexidine has been eluted and record the chromatogram of the test solution for six times the retention time of the peak due to chlorhexidine. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks, other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with a relative retention time of 0.25 or less with respect to the principal peak and any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (c).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.1 g in 5 ml of *anhydrous formic acid* and add 70 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M perchloric acid* is equivalent to 0.01446 g of $C_{22}H_{32}Cl_4N_{10}$.

Chlorhexidine Mouthwash

Chlorhexidine Mouthwash contains Chlorhexidine Gluconate Solution in a suitable flavoured and coloured vehicle.

Chlorhexidine Mouthwash contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chlorhexidine gluconate, $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

Usual strengths. 0.2 per cent w/v; 0.4 per cent w/v; 0.5 per cent w/v; 4 per cent w/v; 20 per cent w/v.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

4-Chloroaniline. Not more than 0.3 per cent.

Determine by gas chromatography (2.4.13).

Test solution (a). Dilute a volume of the mouthwash containing 25 mg of chlorhexidine gluconate to 50 ml with *water*, shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of *0.6 M sodium hydrogen carbonate solution*, allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulphate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µl of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 ml of *0.6 M sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

Test solution (b). Dilute a volume of the mouthwash containing 25 mg of chlorhexidine gluconate to 50 ml with *water*, add 2 ml of reference solution (a), shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of *0.6 M sodium hydrogen carbonate solution* allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulphate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µl of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 ml of *0.6 M sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

Reference solution (a). Dissolve 80 mg of *2,6-dimethylaniline* (internal standard) in 1.0 ml of *1M hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 100.0 ml and dilute 1.0 volume of the solution to 100.0 volumes with *0.01M hydrochloric acid* .

Reference solution (b). Prepare a series of reference solutions in the following manner. Dissolve 25 mg of *4-chloroaniline* in 1 ml of *1M hydrochloric acid* with the aid of ultrasound, add sufficient *water* to produce 200 ml and dilute 1 volume to 10 volumes with the same solvent. To separate 0, 2, 4, 6 and 8 ml volumes of the solution (containing 0, 25, 50, 75 and 100 µg of 4-chloroaniline) add 2 ml of reference solution (a) and sufficient *water* to produce 50 ml, shake with 20 ml of a mixture of 20 volumes of *ether* and 80 volumes of *hexane* and 5 ml of *0.6M sodium hydrogen carbonate solution* allow to separate and discard the aqueous layer. Shake the organic layer with *anhydrous sodium sulphate* and filter through silica treated filter paper (Whatman 1PS is suitable), add 100 µl of *heptafluorobutyric anhydride* and shake for 30 seconds. Allow the solution to stand for 2 minutes, add 5 ml of *0.6 M sodium hydrogen carbonate solution*, shake, allow to separate and use the upper layer.

Chromatographic system
- a glass column 1.5 m x 4.0 mm, packed with acid-washed, silanised diatomaceous support coated with 15 per cent w/w cyanopropylmetylphenyl methyl silicon fluid (Such as OV-225),
- temperature:
  column at 190°,
  inlet port at 200° and detector at 270°,
- electron capture detector,
- flow rate: 50 ml per minute using nitrogen as the carrier gas.

Inject 1 µl the reference solutions and construct a calibration curve of the concentration of 4-chloroaniline against the ratio of the area of the peak corresponding to 4-chloroaniline to the area of the peak corresponding to reference solution (a).

Inject 1 µl test solution (b). Determine the ratio of the area of any peak corresponding to 4-chloroaniline to the area of the peak corresponding to reference solution (a) and hence calculate the content of 4-chloroaniline in the mouthwash with respect to the labelled content of chlorhexidine gluconate.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a quantity of mouthwash with the mobile phase to obtain a solution containing 0.01 per cent w/v of chlorhexidine gluconate.

Reference solution. A 0.008 per cent w/v solution of *chlorhexidine acetate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: dissolve 2.0 g of *sodium octanesulphonate* in a mixture of 120 volumes of *glacial acetic acid*, 270 volumes of *water* and 730 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equillibrate the column atleast for 1 hour.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$ in the mouthwash.

1 mg of chlorhexidine acetate is equivalent to 1.435 mg of $C_{22}H_{30}Cl_2N_{10},2C_6H_{12}O_7$.

Storage. Store protected from light.

Chlorinated Lime

$Ca(ClO)_2$ Mol. Wt. 143.0

Chlorinated Lime contains not less than 30 per cent w/w of available chlorine.

Category. Disinfectant.

Description. An off white powder.

Identification

A. Evolves chlorine copiously on the addition of *2 M hydrochloric acid.*

B. When shaken with *water* and filtered, the filtrate gives reaction (C) of calcium salts and reaction (A) of chlorides (2.3.1).

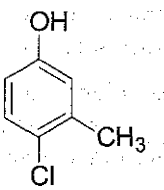
Test

Assay. Triturate 4 g of substance under examination with small quantities of *water* and dilute to 1000 ml with *water;* mix thoroughly. Mix 100 ml of the resulting suspension with a solution containing 3 g of *potassium iodide* in 100 ml of *water*, acidify with 5 ml of *6 M acetic acid* and titrate the liberated iodine with *0.1 M sodium thiosulphate*, using 1 ml of *starch solution* added towards the end of titrations as an indicator.

1.0 ml of *0.1 M sodium thiosulphate* is equivalent to 0.003545 g of available chlorine.

Storage. Store protected from moisture.

Chlorocresol



C_7H_7ClO Mol. Wt. 142.6

Chlorocresol is 4-chloro-3-methylphenol.

Chlorocresol contains not less than 98.0 per cent and not more than 101.0 per cent of C_7H_7ClO.

Category. Antiseptic; pharmaceutical aid (antimicrobial preservative).

Description. Colourless or almost colourless crystals or a white, crystalline powder; volatile in steam.

Identification

A. To a saturated solution in *water* add one drop of *ferric chloride test solution*; a bluish colour is produced.

B. To 0.1 g add 0.2 ml of *benzoyl chloride* and 0.5 ml of *2 M sodium hydroxide*. Shake vigorously until a white precipitate is produced, add 5 ml of *water* and filter. The melting range of the residue, after crystallisation from *methanol* and drying at 70°, is 85° to 88° (2.4.21).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity or alkalinity. To 10 ml of a 5.0 per cent w/v solution add 0.1 ml of *methyl red solution*. The solution is orange or red and not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to yellow.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. A 1.0 per cent w/v solution of the substance under examination in *acetone*.

Chromatographic system
- a glass column 1.8m x 3.5 mm, packed with silanised diatomaceous support (80 to 120 mesh) impregnated with 3 to 5 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (Such as OV-17),
- temperature:
  column.125°,
  inlet port. 210°,
  detector. 230°,
- flow rate: 30 ml per minute using nitrogen as the carrier gas.

Allow the chromatography to proceed for three times the retention time of chlorocresol (about 8 minutes).

Inject 1 µl the test solution. The sum of the areas of all secondary peaks in the chromatogram is not greater than 1.0 per cent of the total area of the peaks.

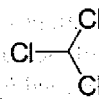
Non-volatile matter. Not more than 0.1 per cent, determined on 2.0 g by volatilising on a water-bath and drying at 105°.

Assay. Weigh accurately about 70 mg, dissolve in 30 ml of *glacial acetic acid*, add 25.0 ml of *0.0167 M potassium bromate*, 20.0 ml of a 15 per cent w/v solution of *potassium bromide* and 10 ml of *hydrochloric acid*. Stopper the flask and allow to stand in the dark for 15 minutes, shaking occasionally. Add 1 g of *potassium iodide* and 100 ml of *water*. Titrate with *0.1 M sodium thiosulphate*, shaking vigorously and using *starch solution*, added towards the end of the titration, as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of potassium bromate required.

1 ml of *0.0167 M potassium bromate* is equivalent to 0.003565 g of C_7H_7ClO.

Storage. Store protected from light and moisture.

Chloroform



$CHCl_3$ Mol. Wt. 119.4

Chloroform is trichloromethane to which either 1.0 per cent to 2.0 per cent v/v of ethanol or 50 mg per litre of amylene has been added.

Category. Pharmaceutical aid (solvent and antimicrobial preservative).

Description. A colourless, volatile liquid.

NOTE — Care should be taken not to vaporise chloroform in the presence of a flame because of the production of harmful gases.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Shake with an equal volume of *water* and dry with *anhydrous sodium sulphate*. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chloroform IPRS* or the spectrum with the reference spectrum of chloroform.

B. Non-flammable. The vapour introduced into a Bunsen flame produces a green colour and gives rise to noxious vapours having a characteristic odour.

C. Warm 0.5 ml with 0.05 ml of *aniline* and 1 ml of *5 M sodium hydroxide*. The characteristic odour of phenyl isocyanide is produced.

Tests

Weight per ml (2.4.29). 1.474 g to 1.478 g.

Boiling range (2.4.8). Not more than 5.0 per cent v/v distils below 60° and the remainder distils between 60° and 62°.

Acidity or alkalinity. Shake 10 ml with 20 ml of freshly boiled and cooled *water* for 3 minutes and allow to separate. To 5 ml of the aqueous layer (solution A) add 0.1 ml of *litmus solution*; the

colour produced is similar to that produced on adding 0.1 ml of *litmus solution* to 5 ml of freshly boiled and cooled *water*.

Chlorides. To 5 ml of solution A add 5 ml of *water* and 0.2 ml of *silver nitrate solution*; the solution is *clear*.

Free chlorine. To 10 ml of solution A add 1 ml of *cadmium iodide solution* and 2 drops of *starch solution*; no blue colour is produced.

Aldehyde. Shake 5 ml with 5 ml of *water* and 0.2 ml of *alkaline potassium mercuri-iodide solution* in a stoppered bottle and set aside in the dark for 15 minutes; not more than a pale yellow colour is produced.

Foreign chlorine compounds. Shake 20 ml with 10 ml of *sulphuric acid* in a stoppered flask for 5 minutes, allow to stand in the dark for 30 minutes and discard the acid layer. Shake 15 ml of the chloroform layer with 30 ml of *water* in a stoppered flask for 3 minutes and allow to separate. To the aqueous layer add 0.2 ml of *silver nitrate solution* and set aside in the dark for 5 minutes; no opalescence is produced.

Related substances. Determine by gas chromatography (2.4.13).

Test solution (a). A solution containing 0.2 per cent v/v of *carbon tetrachloride*, 0.2 per cent v/v of *1,1,1-trichloroethane* (internal standard), 0.2 per cent v/v of *dichloromethane*, 0.2 per cent v/v of *ethanol*, 0.5 per cent v/v of *bromochloromethane* and 0.2 per cent v/v of the substance under examination in *1-propanol*.

Test solution (b). The substance under examination.

Reference solution (a). A solution containing 0.2 per cent v/v of the internal standard in the substance under examination.

Reference solution (b). *1-propanol*.

Chromatographic system

- a glass column 4 m x 3 mm, packed with acid-washed kieselguhr (60 to 100 mesh) coated with 15 per cent w/w of *di-2-cyanoethyl ether*,
- temperature:
  column 40°,
  inlet port and detector 100°,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas,
- inject 0.1 µl of each solution.

The test is not valid unless the column efficiency, determined using the chloroform peak in the chromatogram obtained with test solution (a), is greater than 700 plates per metre and the total number of plates is greater than 2,500.

In the chromatogram obtained with test solution (a) the peaks, in the order of emergence, are due to carbon tetrachloride, 1,1,1-trichloroethane, dichloromethane, chloroform, ethanol, bromochloromethane and 1-propanol (solvent).

Using the chromatogram obtained with reference solution (b) make any corrections due to the contribution of secondary peaks from the solvent to the peaks in the chromatogram obtained with test solution (a).

In the chromatogram obtained with reference solution (a), the ratio of the areas of any peaks due to carbon tetrachloride, dichloromethane and bromochloromethane to the area of the peak due to the internal standard is not more than the corresponding ratios in the chromatogram obtained with test solution (a) and the ratio of the area of any other secondary peak that elutes prior to the solvent peak, except for the peak corresponding to ethanol, to the area of the peak due to the internal standard is not more than the ratio of the area of the peak due to chloroform to the area of the peak due to the internal standard in the chromatogram obtained with test solution (a).

Calculate the content of each of the specified impurities and also calculate the content of each of any other impurities assuming the same response per unit volume as with chloroform. The total content of all impurities is not more than 1.0 per cent v/v.

Ethanol *(if present)*. Determine by gas chromatography (2.4.13).

Test solution (a). The substance under examination.

Test solution (b). A solution containing 1.0 per cent v/v of *1-propanol* (internal standard) in the substance under examination.

Reference solution. A solution containing 1.0 per cent v/v of *ethanol* and 1.0 per cent v/v of the internal standard in *water*.

Inject 0.1 µl of each solution.

Use the chromatographic procedure described under Related substances.

The test is not valid unless the height of the trough separating the ethanol peak from the chloroform peak in the chromatogram obtained with test solution (a) is less than 15 per cent of the height of the ethanol peak.

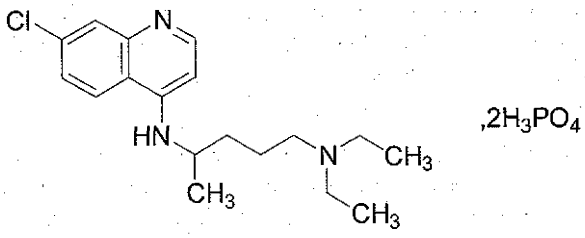
Calculate the content of ethanol from the areas of the peaks due to ethanol and the internal standard in the chromatograms obtained with reference solution and test solution (b).

Non-volatile matter. Not more than 0.004 per cent w/v, determined on 25 ml by evaporation to dryness and drying at 105°.

Storage. Store protected from light in tightly-closed, glass-stoppered containers.

Labelling. The label states whether it contains ethanol or amylene.

Chloroquine Phosphate



$C_{18}H_{26}ClN_3,2H_3PO_4$ Mol. Wt. 515.9

Chloroquine Phosphate is (*RS*)-7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline diphosphate.

Chloroquine Phosphate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{26}ClN_3,2H_3PO_4$, calculated on the anhydrous basis.

Category. Antimalarial; antiamoebic.

Description. A white or almost white, crystalline powder. It slowly gets discoloured on exposure to light. It may exist in two polymorphic forms differing in their behaviour, one of which melts at about 195° and the other at about 218°.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of *chloroquine phosphate IPRS* treated in the same manner or with the reference spectrum of chloroquine.

B. When examined in the range 210 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution shows absorption maxima at about 220 nm, 235 nm, 256 nm, 329 nm and 342 nm; absorbance at about 220 nm, 0.60 to 0.66, at about 235 nm, 0.35 to 0.39, at about 256 nm, 0.30 to 0.33, at about 329 nm, 0.325 to 0.355 and at about 342 nm, 0.36 to 0.39.

C. Dissolve 25 mg in 20 ml of *water* and add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at 205° to 210° (2.4.21).

D. Neutralise with *dilute nitric acid* the aqueous layer obtained in test A. Add an equal volume of *ammonium molybdate solution* and warm; a yellow precipitate is produced.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BYS5 or GYS5 (2.4.1).

pH (2.4.24). 3.5 to 4.5, determined in a 10.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine*.

Test solution. A 5.0 per cent w/v solution of the substance under examination in *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

Reference solution (b). Dilute 25 ml of reference solution (a) to 50.0 ml with *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Water (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 15 mg of the substance under examination in *water* and dilute to 100.0 ml with *water*.

Reference solution (a). A 0.015 per cent w/v solution of *chloroquine phosphate IPRS* in *water*.

Reference solution (b). A solution containing 0.015 per cent w/v of *chloroquine phosphate IPRS* and 0.0015 per cent w/v of *hydroxychloroquine sulphate IPRS* in *water*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 78 volumes of buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000.0 ml of *water*, add 1.0 ml of *perchloric acid*, adjusted to pH 2.5 with *orthophosphoric acid* and 22 volumes of *methanol*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 224 nm,
- injection volume: 10 µl.

The relative retention time with reference to chloroquine phosphate for hydroxychloroquine sulphate is about 0.8.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to chloroquine phosphate and hydroxychloroquine sulphate is not less than 1.5. The column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{26}ClN_3,2H_3PO_4$.

Storage. Store protected from light.

Chloroquine Phosphate Injection

Chloroquine Phosphate Injection is a sterile solution of Chloroquine Phosphate in Water for Injections.

Chloroquine Phosphate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, $C_{18}H_{26}ClN_3$.

Usual strength. The equivalent of 40 mg of chloroquine per ml. (250 mg of chloroquine phosphate is approximately equivalent to 155 mg of chloroquine).

Description. A clear, colourless or almost colourless solution.

Identification

A. To a volume of the injection containing 60 mg of chloroquine add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of *chloroquine phosphate IPRS* treated in the same manner or with the reference spectrum of chloroquine.

B. Dilute a volume of the injection containing 15 mg of chloroquine to 20 ml with *water* and add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at about 207° (2.4.21).

C. Neutralise the aqueous layer obtained in test A with *dilute nitric acid*, add an equal volume of *ammonium molybdate solution* and warm; a yellow precipitate is produced.

Tests

pH (2.4.24). 3.5 to 4.5.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume of the injection containing 0.4 g of chloroquine add 20 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid* and mix. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01599 g of chloroquine, $C_{18}H_{26}ClN_3$.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of chloroquine in a suitable dose-volume.

Chloroquine Phosphate Suspension

Chloroquine Phosphate Suspension is a suspension of Chloroquine Phosphate in a suitable flavoured vehicle.

Chloroquine Phosphate Suspension contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, $C_{18}H_{26}ClN_3$.

Usual strength. The equivalent of 50 mg of chloroquine in 5 ml. (80 mg of chloroquine phosphate is approximately equivalent to 50 mg of chloroquine).

Identification

To a volume of the suspension containing 50 mg of chloroquine add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of *chloroquine phosphate IPRS* treated in the same manner or with the reference spectrum of chloroquine.

Tests

pH (2.4.24). 5.5 to 6.5.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Weigh accurately a quantity of the suspension containing about 100 mg of chloroquine, add 50 ml of *1 M hydrochloric acid*, shake well and dilute to 100.0 ml with *1 M hydrochloric acid*. Filter and discard the first few ml of the

filtrate. Dilute 10.0 ml of the filtrate to 100.0 ml with *1 M hydrochloric acid* and mix. Further dilute 10.0 ml to 100.0 ml with the same solvent and mix. Measure the absorbance of the resulting solution at the maximum at about 342 nm (2.4.7). Calculate the content of $C_{18}H_{26}ClN_3$ from the absorbance obtained by repeating the operation using *chloroquine phosphate IPRS* in place of the substance under examination.

Chloroquine Phosphate Tablets

Chloroquine Phosphate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chloroquine phosphate, $C_{18}H_{26}ClN_3,2H_3PO_4$. The tablets are coated.

Usual strength. 250 mg. (250 mg of chloroquine phosphate is approximately equivalent to 155 mg of chloroquine).

Identification

A. To a quantity of the powdered tablets containing 0.1 g of Chloroquine Phosphate add 10 ml of *water* and 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 80 mg of *chloroquine phosphate IPRS* treated in the same manner or with the reference spectrum of chloroquine.

B. Extract a quantity of the powdered tablets containing 25 mg of Chloroquine Phosphate with 20 ml of *water*, filter and to the filtrate add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at about 207° (2.4.21).

C. Extract a quantity of the powdered tablets containing 0.5 g of Chloroquine Phosphate with 25 ml of *water* and filter. To the filtrate add 2.5 ml of *5 M sodium hydroxide* and extract with three quantities, each of 10 ml, of *ether*. The aqueous layer, after neutralisation with *2 M nitric acid*, gives the reactions of phosphates (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 344 nm (2.4.7). Calculate the content of $C_{18}H_{26}ClN_3,2H_3PO_4$ in the medium taking 371 as the specific absorbance at 344 nm.

Q. Not less than 70 per cent of the stated amount of $C_{18}H_{26}ClN_3,2H_3PO_4$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine*.

Test solution. Shake a quantity of the powdered tablets containing 1.0 g of Chloroquine Phosphate with 20.0 ml of *water* for 30 minutes, centrifuge and use the clear, supernatant liquid.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

Reference solution (b). Dilute 25.0 ml of reference solution (a) to 50.0 ml with *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 15 mg of Chloroquine Phosphate in *water* and dilute to 100.0 ml with *water*, with the aid of ultrasound for 20 minutes and filter.

Reference solution (a). A 0.015 per cent w/v solution of *chloroquine phosphate IPRS* in *water*.

Reference solution (b). A solution containing 0.015 per cent w/v of *chloroquine phosphate IPRS* and 0.0015 per cent w/v of *hydroxychloroquine sulphate IPRS* in *water*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 78 volumes of buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000.0 ml of *water*, add 1.0 ml of *perchloric acid* and adjusted to pH 2.5 with *orthophosphoric acid* and 22 volumes of *methanol*,

– flow rate: 1.2 ml per minute,
– spectrophotometer set at 224 nm,
– injection volume: 10 µl.

The relative retention time with reference to chloroquine phosphate for hydroxychloroquine sulphate is about 0.8.

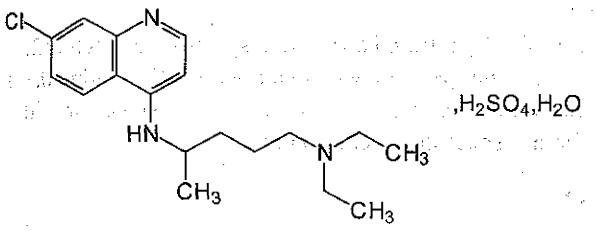
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to chloroquine phosphate and hydroxychloroquine sulphate is not less than 1.5. The tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{26}ClN_3,2H_3PO_4$ in the tablets.

Storage. Store protected from light.

Chloroquine Sulphate



$C_{18}H_{26}ClN_3,H_2SO_4,H_2O$ Mol. Wt. 435.9

Chloroquine Sulphate is *(RS)*-4-(7-chloro-4- quinolyl-amino) pentyldiethylamine sulphate monohydrate.

Chloroquine Sulphate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{26}ClN_3,H_2SO_4$, calculated on the anhydrous basis.

Category. Antimalarial; antiamoebic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate IPRS* in the same manner or with the reference spectrum of chloroquine.

B. When examined in the range 210 nm to 360 nm, a 0.001 per cent w/v solution shows absorption maxima at about 220 nm, 235 nm, 256 nm, 329 nm and 342 nm; absorbance at about 220 nm, 0.73 to 0.81, at about 235 nm, 0.43 to 0.47, at about 256 nm, 0.37 to 0.41, at about 329 nm, 0.40 to 0.44 and at about 342 nm, 0.43 to 0.47 (2.4.7).

C. Dissolve 25 mg in 20 ml of *water* and add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at 205° to 210° (2.4.21).

D. Gives reaction A of sulphates (2.3.1).

Tests

Appearance of solution. An 8.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BYS5 or GYS5 (2.4.1).

pH (2.4.24). 4.0 to 5.0, determined in an 8.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine*.

Test solution. A 5.0 per cent w/v solution of the substance under examination in *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

Reference solution (b). Dilute 25.0 ml of reference solution (a) to 50.0 ml with *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g dissolved in 25 ml of *water* complies with the limit test for heavy metals, Method A (20 ppm).

Chlorides (2.3.12). 1.25 g complies with the limit test for chlorides (200 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 3.0 to 5.0 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.5 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0418 g of $C_{18}H_{26}ClN_3,H_2SO_4$.

Storage. Store protected from light.

Chloroquine Sulphate Injection

Chloroquine Sulphate Injection is a sterile solution of Chloroquine Sulphate in Water for Injections.

Chloroquine Sulphate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, $C_{18}H_{26}ClN_3$.

Usual strength. The equivalent of 40 mg of chloroquine per ml. (200 mg of chloroquine sulphate is approximately equivalent to 147 mg of chloroquine).

Description. A clear, colourless or almost colourless solution.

Identification

A. To a volume of the injection containing 70 mg of chloroquine add sufficient *water* to produce 10 ml, add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate IPRS* in the same manner or with the reference spectrum of chloroquine.

B. When examined in the range 210 nm to 360 nm, a 0.001 per cent w/v solution shows absorption maxima at about 220 nm, 235 nm, 256 nm, 329 nm and 342 nm; absorbance at about 220 nm, 0.73 to 0.81, at about 235 nm, 0.43 to 0.47, at about 256 nm, 0.37 to 0.41, at about 329 nm, 0.40 to 0.44 and at about 342 nm, 0.43 to 0.47 (2.4.7).

C. It gives reaction (A) of sulphates (2.3.1).

Tests

pH (2.4.24). 4.0 to 5.5.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume of the injection containing 0.4 g of chloroquine add 20 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid* and mix. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml *0.1 M perchloric acid* is equivalent to 0.01599 g of $C_{18}H_{26}ClN_3$.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of chloroquine in a suitable dose-volume.

Chloroquine Syrup

Chloroquine Syrup is a solution of Chloroquine Phosphate or Chloroquine Sulphate in a suitable flavoured vehicle.

Chloroquine Syrup contains Chloroquine Phosphate or Chloroquine Sulphate equivalent to not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloroquine, $C_{18}H_{26}ClN_3$.

Usual strength. The equivalent of 50 mg of chloroquine in 5 ml. (80 mg of Chloroquine Phosphate or 67 mg of Chloroquine Sulphate is approximately equivalent to 50 mg of chloroquine).

Identification

To a volume of the syrup containing 50 mg of chloroquine add 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with water, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate IPRS* in the same manner or with the reference spectrum of chloroquine.

Tests

pH (2.4.24). 4.0 to 6.5.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. To an accurately measured volume of the syrup containing about 0.4 g of chloroquine add 20 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid* and mix. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01599 g of $C_{18}H_{26}ClN_3$.

Storage. Store protected from light.

Labelling. The label states (1) whether the syrup contains Chloroquine Phosphate or Chloroquine Sulphate; (2) the strength in terms of equivalent amount of chloroquine in each 5 ml.

Chloroquine Sulphate Tablets

Chloroquine Sulphate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chloroquine sulphate, $C_{18}H_{26}ClN_3,H_2SO_4$. The tablets are coated.

Usual strength. 200 mg. (200 mg of chloroquine sulphate is approximately equivalent to 147 mg of chloroquine).

Identification

A. To a quantity of the powdered tablets equivalent to 0.1 g of Chloroquine Sulphate add 10 ml of *water* and 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by treating 0.1 g of *chloroquine sulphate IPRS* in the same manner or with the reference spectrum of chloroquine.

B. Extract a quantity of the powdered tablets containing 25 mg of Chloroquine Sulphate with 20 ml of *water*, filter and to the filtrate add 8 ml of *picric acid solution*; the precipitate, after washing successively with *water*, *ethanol (95 per cent)* and *ether*, melts at about 207° (2.4.21).

C. Extract a quantity of the powdered tablets containing about 0.1 g of Chloroquine Sulphate with 10 ml of *water* and 1 ml of *dilute hydrochloric acid* and filter. To the filtrate add 1 ml of *barium chloride solution*; a white precipitate is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 344 nm (2.4.7). Calculate the content of $C_{18}H_{26}ClN_3,H_2SO_4$ in the medium taking 450 as the specific absorbance at 344 nm.

Q. Not less than 70 per cent of the stated amount of $C_{18}H_{26}ClN_3,H_2SO_4$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica GF254*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 40 volumes of *cyclohexane* and 10 volumes of *diethylamine*.

Test solution. Shake a quantity of powdered tablets containing about 2.0 g of Chloroquine Sulphate with 50 ml of *water* for 30 minutes, centrifuge and use the supernatant liquid, if necessary filter through suitable filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

Reference solution (b). Dilute 25.0 ml of reference solution (a) to 50.0 ml with *water*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

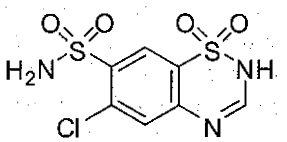
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.5 g of Chloroquine Sulphate, add 20 ml of *1 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid* and mix. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0209 g of $C_{18}H_{26}ClN_3,H_2SO_4$.

Storage. Store protected from light.

Chlorothiazide



$C_7H_6ClN_3O_4S_2$ Mol. Wt. 295.7

Chlorothiazide is 6-chloro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide

Chlorothiazide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_6ClN_3O_4S_2$, calculated on the dried basis.

Category. Diuretic; antihypertensive.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorothiazide IPRS* or with the reference spectrum of chlorothiazide.

B. Dissolve 80 mg in 100 ml of *0.1 M sodium hydroxide* and dilute to 1000.0 ml with *water*. Dilute 10.0 ml of the solution to 100.0 ml with *0.01 M sodium hydroxide*. When examined in the range 220 nm to 320 nm (2.4.7), shows two absorption maxima at about 225 nm and 292 nm. The specific absorbance at the maxima are 725 to 800 and 425 to 450 respectively.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Ethyl acetate.

Test solution. Dissolve 25 mg of the substance under examination in 5.0 ml of *acetone*.

Reference solution. A 0.5 per cent w/v solution of *chlorothiazide IPRS* in *acetone*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

D. To 0.1 g, add a pellet of *sodium hydroxide* and heat strongly. Gas is evolved which turns *red litmus paper* to blue. After cooling, take up the residue with 10 ml of *dilute hydrochloric acid*. Gas is evolved which turns *lead acetate paper* to black.

Tests

Solution A. Dissolve 1.0 g of the substance under examination in 50 ml of *water*.

Acidity or alkalinity. To 10 ml of solution A, add 0.2 ml of *0.01 M sodium hydroxide* and 0.15 ml of *methyl red solution*. The solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Related substances. Determine by thin-layer chromatography (2:4.17) coating the plate with *silica gel G*.

Mobile phase. A mixture of 15 volumes of *2-propanol* and 85 volumes of *ethyl acetate*.

Test solution. Dissolve 25 mg of the substance under examination in 5.0 ml of *acetone*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *acetone*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with a mixture of equal volumes of *alcoholic solution of sulphuric acid* and *alcohol*. Heat the plate at 105° for 30 minutes and immediately place the plate in the tank having 10 ml of a saturated solution of *sodium nitrite* in a beaker. Carefully add 0.5 ml of *sulphuric acid* to the *sodium nitrite solution*, close the tank, and allow to stand for 15 minutes. Remove the plate, heat in a ventilated oven at 40° for 15 minutes and spray with three quantities, each of 5 ml, of a freshly prepared 0.5 per cent w/v solution of *naphthylethylenediamine dihydrochloride* in *alcohol*. Examine the plate in day light. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Chlorides (2.3.12). Dissolve 1.5 g in 15 ml of *water*, filter. The solution complies with the limit test for chlorides (160 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.25 g in 50 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide* in *2-propanol* to the first point of inflexion. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02957 g of $C_7H_6ClN_3O_4S_2$.

Chlorothiazide Oral Suspension

Chlorothiazide Oral Suspension is a dry mixture of Chlorothiazide with buffering agent and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the content of the sealed container in the specified volume of water just before use.

Chlorothiazide Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorothiazide, $C_7H_6ClN_3O_4S_2$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of chlorothiazide, $C_7H_6ClN_3O_4S_2$.

Usual strength. 50 mg per ml.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

The constituted suspension complies with the tests stated under Oral Liquids and with the following tests.

Identification

When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the assay shows an absorption maxima

at the same wavelength as that of solution of *chlorothiazide IPRS* prepared in the same manner.

Tests

pH (2.4.24). 3.2 to 4.0, determined on constituted solution.

Other tests. Comply with tests stated under Oral Suspension.

Assay. Weigh accurately a quantity of the suspension containing about 0.25 g of chlorothiazide, diluted to 250.0 ml with *sodium hydroxide solution* (1 in 250) and mix. Dilute 10.0 ml of the solution to 100.0 ml with *diluted hydrochloric acid* (1 in 100) and mix. Transfer 50.0 ml of the resulting solution to a 125-ml separator, and wash with two, 25 ml portions of *chloroform*, discarding the washing. Dilute 10.0 ml of the washed solution to 100 ml with *sodium hydroxide solution* (1 in 250) and mix. Dissolve an accurately weighed quantity of *chlorothizide IPRS* in *sodium hydroxide solution* (1 in 250) to obtain a concentration of about 10 µg per ml and measure the absorbance of the both solutions at the maxima (2.4.7) at about 292 nm.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of chlorothiazide $C_7H_6ClN_3O_4S_2$ weight in oral suspension.

Chlorothiazide Tablets

Chlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorothiazide, $C_7H_6ClN_3O_4S_2$.

Usual strengths. 250 mg; 500 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Gives the reaction of sulphite (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 8.0*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Use the filtrate, dilute if necessary, with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 294 nm (2.4.7). Calculate the content of $C_7H_6ClN_3O_4S_2$ in the medium from the absorbance obtained from a solution of known concentration of *chlorothiazide IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_7H_6ClN_3O_4S_2$.

Other tests. Comply with tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Weigh and powder 20 Tablets. Disperse a quantity of the powder containing about 0.25 g of Chlorothiazide with 50.0 ml of *0.05 M monobasic sodium phosphate buffer*, shake for 15 minutes and add 100.0 ml of *acetonitrile*, dilute to 500.0 ml with *water*, filter.

Reference solution. Dissolve 25 mg of *chlorothiazide IPRS* in 5.0 ml of *0.05 M monobasic sodium phosphate buffer*, add 10.0 ml of *acetonitrile* and dilute to 50.0 ml with *water*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 95 volumes of *0.08 M monobasic sodium phosphate*, adjusted to pH 2.9 with *orthophosphoric acid* and 5 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

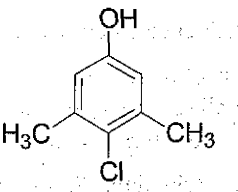
Inject the reference solution. The test is not valid unless the column efficiency is not less than 1300 theoretical plates, the capacity factor is not less than 4.3. The tailing factor is not less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_7H_6ClN_3O_4S_2$ in the tablets.

Storage. Store protected from moisture.

Chloroxylenol



C_8H_9ClO Mol. Wt. 156.6

Chloroxylenol is 4-chloro-3,5-dimethylphenol.

Chloroxylenol contains not less than 98.0 per cent and not more than 103.0 per cent of C_8H_9ClO.

Category. Antiseptic; disinfectant.

Description. A white or creamy-white crystals or crystalline powder. It is volatile in steam.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chloroxylenol IPRS* or with the reference spectrum of chloroxylenol.

B. Dissolve 0.1 g in 5 ml of *chloroform* and add 0.5 ml of filtered 1 per cent w/v solution of *ferric chloride* in *chloroform* and 0.1 ml of *pyridine*; a blue colour is produced.

C. To 5 ml of a saturated solution in *water* add 0.5 ml of *ferric chloride test solution*; no blue colour is produced.

D. Mix 50 mg with 0.5 g of *anhydrous sodium carbonate* and ignite strongly, cool, boil the residue with 5 ml of *water*, acidify with *nitric acid*, filter and add 2 ml of *silver nitrate solution*; a white precipitate is produced.

Tests

Related substances. Determine by gas chromatography (2.4.13).

Test solution. A 2.0 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution. A solution containing 2.0 per cent w/v of the substance under examination and 0.04 per cent w/v of *4-chloro-o-cresol* (internal standard) in chloroform.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of polyethylene glycol (Such as Carbowax 20M),
- temperature:
  column 160°,
  inlet port and detector. 220°,
- a flame ionisation detector,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of the reference solution and the test solution.

In the chromatogram obtained with the reference solution the sum of the areas of all secondary peaks is not greater than the area of the peak due to internal standard.

Assay. Weigh accurately about 70 mg, dissolve in 30 ml of *glacial acetic acid*, add 25.0 ml of *0.0167 M potassium bromate*, 20 ml of 15 per cent w/v solution of *potassium bromide* and 10 ml of *hydrochloric acid*, stopper the flask and allow to stand protected from light for 15 minutes. Add 1 g of *potassium iodide* and 100 ml of *water* and titrate with *0.1 M sodium thiosulphate*, shaking vigorously and using 1 ml of *starch solution* as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *potassium bromate* required.

1 ml of *0.0167 M potassium bromate* is equivalent to 0.003915 g of C_8H_9ClO.

Chloroxylenol Solution

Chloroxylenol solution is a solution of Chloroxylenol solubilised in a saponaceous base containing Ethanol (95 per cent) and essential oils. Ethanol (95 per cent) may be replaced by Industrial Methylated Spirit in making Chloroxylenol Solution.

Chloroxylenol Solution contains not less than 4.75 per cent and not more than 5.25 per cent of C_8H_9ClO.

Usual strength. 5 per cent w/v.

Tests

pH (2.4.24). 7.0 to 11.0.

Ethanol content (2.3.45). 16 to 21 per cent v/v.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Extract 4 ml of the solution under examination with 20.0 ml of *chloroform* after adding 4 ml of *2 M hydrochloric acid*. Extract with two further quantities, each of 10.0 ml, of *chloroform*. Combine the chloroform extracts, shake with *anhydrous sodium sulphate* and filter.

Reference solution (a). Dissolve 0.1 g of *chloroxylenol IPRS* in 10.0 ml of a 0.8 per cent w/v solution of *4-chloro-o-cresol* (internal standard) in *chloroform* (solution A) and dilute to 20.0 ml with *chloroform*.

Reference solution (b). Prepare in the same manner as the test solution but use 20.0 ml of solution A instead of 20 ml of *chloroform*.

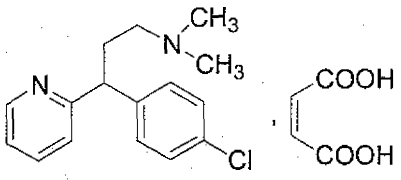
Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of polyethylene glycol (Such as Carbowax 20M),
- temperature:
  column 160°,
  inlet port and detector. 220°,
- a flame ionisation detector,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of reference solution (a), (b) and the test solution.

Calculate the content of C_8H_9ClO in the solution.

Labelling. The label states that the preparation is meant for external use only.

Chlorpheniramine Maleate



$C_{16}H_{19}ClN_2,C_4H_4O_4$ Mol. Wt. 390.9

Chlorpheniramine Maleate is (*RS*)-3-(4-chlorophenyl)-3-(pyrid-2-yl)propyldimethylamine hydrogen maleate.

Chlorpheniramine Maleate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{16}H_{19}ClN_2,C_4H_4O_4$, calculated on the dried basis.

Category. Antihistaminic.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorpheniramine maleate IPRS* or with the reference spectrum of chlorpheniramine maleate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 10.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 4.0 to 5.0, determined in a 1.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *diethylamine*.

Test solution. A 5.0 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *chloroform* and mix. Dilute 5.0 ml of the resulting solution to 25.0 ml with *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 with the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *chlorpheniramine maleate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- column temperature: 25°,
- mobile phase: a mixture of 20 volumes of *acetonitrile* and 80 volumes of a buffer solution prepared by dissolving 8.57 g of *ammonium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and filter,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

The retention time of principal peak is about 11 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$.

Storage. Store protected from light and moisture.

Chlorpheniramine Injection

Chlorpheniramine Maleate Injection

Chlorpheniramine Injection is a sterile solution of Chlorpheniramine Maleate in Water for Injections free from dissolved air and containing suitable buffering and stabilising agents.

Chlorpheniramine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlorpheniramine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$.

Usual strength. 10 mg in 1 ml.

Description. A colourless solution.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. Heat the plate at 105° for 30 minutes before use.

Mobile phase. A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *methanol* and 20 volumes of *1 M acetic acid*.

Test solution. Evaporate an appropriate volume of the injection to dryness in a current of nitrogen using the minimum amount of heat, dissolve the residue as completely as possible in sufficient *chloroform* to produce a solution containing 0.5 per cent w/v of Chlorpheniramine Maleate and centrifuge.

Reference solution. A 0.5 per cent w/v solution of *chlorpheniramine maleate IPRS* in *chloroform*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution. Spray the plate with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.0 to 5.2.

Related substances. Carry out the method described under the Identification test using as the test solution a solution prepared in the following manner. Evaporate an appropriate volume of the injection to dryness in a current of nitrogen using the minimum amount of heat. Dissolve the residue in sufficient *chloroform* to produce a solution containing 5.0 per cent w/v of Chlorpheniramine Maleate and centrifuge. For the reference solution, dilute 1 volume of the test solution to 500 volumes with *chloroform*. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Bacterial endotoxins (2.2.3). Not more than 8.8 Endotoxin Units per mg of chlorpheniramine maleate.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute an accurately measured volume of the injection containing 10 mg of Chlorpheniramine Maleate to 500.0 ml with *0.25 M sulphuric acid*. Measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ taking 212 as the specific absorbance at 265 nm.

Storage. Store protected from light.

Chlorpheniramine Tablets

Chlorpheniramine Maleate Tablets

Chlorpheniramine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chlorpheniramine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$.

Usual strengths. 4 mg; 8 mg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. Heat the plate at 105° for 30 minutes before use.

Mobile phase. A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *methanol* and 20 volumes of *1 M acetic acid*.

Test solution. Extract a quantity of the powdered tablets containing 5 mg of Chlorpheniramine Maleate with *chloroform*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*.

Reference solution. A 0.5 per cent w/v solution of *chlorpheniramine maleate IPRS* in *chloroform*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The two principal spots obtained in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution. Spray the plate with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate suitably diluted if necessary, at the maximum at about 265 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ in the medium from the absorbance obtained from a solution of known concentration of *chlorpheniramine maleate IPRS* in the same medium.

Q. Not less than 80 per cent of the stated amount of $C_{16}H_{19}ClN_2,C_4H_4O_4$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *diethylamine*.

Test solution. Extract a quantity of the powdered tablets containing 100 mg of Chlorpheniramine Maleate with *chloroform*, filter, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*.

Reference solution. Dilute 1.0 ml of the test solution to 50.0 ml with *chloroform* and dilute 1.0 ml of the resulting solution to 10.0 ml with the same solvent.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

Uniformity of content. Complies with test stated under Tablets.

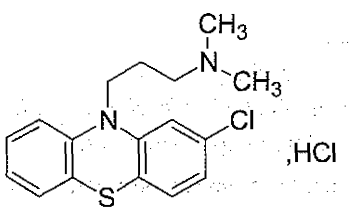
Powder one tablet and carry out the Assay beginning at the words "shake with 20 ml of *0.05 M sulphuric acid*...". Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 4 mg of Chlorpheniramine Maleate, shake with 20 ml of *0.05 M sulphuric acid* for 5 minutes, add 20 ml of *ether*, shake carefully and filter the acid layer into a second separator. Extract the ether layer with two quantities, each of 10 ml, of *0.05 M sulphuric acid*, filter each acid layer into the second separator and wash the filter with *0.05 M sulphuric acid*. Make the combined acid extracts and washing just alkaline to *litmus paper* with *1 M sodium hydroxide*, add 2 ml in excess, and extract with two quantities, each of 50 ml, of ether. Wash each ether extract with the same 20 ml of *water* and extract in succession with 20, 20 and 5 ml of *0.25 M sulphuric acid*, dilute the combined acid extracts to 50.0 ml with *0.25 M sulphuric acid*; dilute 10.0 ml to 50.0 ml with *0.25 M sulphuric acid* and measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$, taking 212 as the specific absorbance at 265 nm.

Storage. Store protected from light and moisture.

Chlorpromazine Hydrochloride



$C_{17}H_{19}ClN_2S,HCl$ Mol. Wt. 355.3

Chlorpromazine Hydrochloride is 2-chloro-10-(3-dimethylaminopropyl)phenothiazine hydrochloride.

Chlorpromazine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{19}ClN_2S,HCl$, calculated on the dried basis.

Category. Antipsychotic; antiemetic.

Description. A white or creamy-white, crystalline powder. It decomposes on exposure to air and light becoming yellow, pink and finally violet.

Identification

Test A may be omitted if tests B, C and D are carried out. Test B may be omitted if tests A, C and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorpromazine hydrochloride IPRS* or with the reference spectrum of chlorpromazine hydrochloride.

B. When examined in the range 230 nm to 360 nm, a 0.0005 per cent w/v solution in *0.1 M hydrochloric acid* shows absorption maxima at about 254 nm and 306 nm, 0.45 to 0.48 (2.4.7).

C. Complies with the test for identification of phenothiazines (2.3.3)

D. A 5 per cent w/v solution gives reaction (B) of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 4.5, determined in a 10.0 per cent solution.

Related substances. Complies with the test for Related substances in Phenothiazines (2.3.5), using mobile phase (a).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.6 g, dissolve in 200 ml of *acetone* and add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03553 g of $C_{17}H_{19}ClN_2S,HCl$.

Storage. Store protected from light and moisture.

Chlorpromazine Injection

Chlorpromazine Hydrochloride Injection

Chlorpromazine Injection is a sterile solution of Chlorpromazine hydrochloride in Water for Injections free from air and containing buffering and stabilizing agents.

Chlorpromazine Hydrochloride contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$.

NOTE — Protect the solutions from light throughout the tests.

Usual strength. 25 mg per ml.

Description. A colourless or almost colourless solution.

Identification

A. To a volume containing 0.1 g of Chlorpromazine Hydrochloride, add 20 ml *of water* and 2 ml *of 10 M sodium hydroxide*. Extract with 25 ml *of ether*, wash the ether extract with two quantities, each of 5 ml, of *water*, dry the ether extract with *anhydrous sodium sulphate*, evaporate the ether and dissolve the residue in 1 ml of *chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorpromazine hydrochloride IPRS* treated in the same manner or with the reference spectrum of chlorpromazine.

B. Dilute a volume of the injection with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.0005 per cent w/v of Chlorpromazine Hydrochloride. The resulting solution, when examined in the range 230 nm to 360 nm shows absorption maxima at about 254 nm and 306 nm, 0.45 to 0.48 (2.4.7).

C. It gives reaction (B) of chlorides (2.3.1).

Tests

Related substances. Complies with the test for Related substances in Phenothiazines (2.3.5), using mobile phase (a) and the following solution.

Test solution. Dilute a volume of the injection with sufficient of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* to produce a solution containing 2.0 per cent of Chlorpromazine Hydrochloride.

Bacterial endotoxins (2.2.3). Not more than 6.9 Endotoxin Units per mg of chlorpromazine hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute an accurately measured volume of the injection with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.0005 per cent w/v of Chlorpromazine Hydrochloride and measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of $C_{17}H_{19}ClN_2S,HCl$, taking 915 as the specific absorbance at 254 nm.

Storage. Store protected from light.

Chlorpromazine Tablets

Chlorpromazine Hydrochloride Tablets

Chlorpromazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S,HCl$. The tablets are coated.

NOTE — Protect the solutions from light throughout the tests.

Usual strengths. 10 mg; 25 mg; 50 mg; 100 mg; 200 mg.

Identification

A. To a quantity of the powdered tablets containing 40 mg of Chlorpromazine Hydrochloride add 10 ml of *water* and 2 ml of *10 M sodium hydroxide*. Extract with 15 ml of *ether* and wash the ether extract with two quantities each of 5 ml, *of water*, dry with *anhydrous sodium sulphate*. Evaporate the ether and dissolve the residue in 0.4 ml *of chloroform*. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorpromazine hydrochloride IPRS* treated in the same manner or with the reference spectrum of chlorpromazine.

B. Digest a quantity of the powdered tablets containing 25 mg of Chlorpromazine Hydrochloride with 25 ml of *water* and filter. Reserve a portion of the filtrate for Identification C. Dilute a volume of the filtrate with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.0005 per cent w/v of Chlorpromazine Hydrochloride. The resulting solution, when examined in the range 230 nm to 360 nm shows absorption maxima at about 254 nm and 306 nm, 0.45 to 0.48 (2.4.7).

C. The filtrate reserved in test B gives reaction B of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium: 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium, if necessary. Measure the absorbance of the

resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of chlorpromazine hydrochloride, $C_{17}H_{19}ClN_2S$, HCl in the medium from the absorbance obtained from a solution of known concentration of *chlorpromazine hydrochloride IPRS* in the dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{19}ClN_2S$, HCl.

Related substances. Complies with the test for Related substances in Phenothiazines (2.3.5), using mobile phase (a) and the following solutions.

Test solution. Extract a quantity of the powdered tablets containing 0.2 g of Chlorpromazine Hydrochloride with 10 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and filter.

Reference solution. Dilute 1 volume of the test solution to 200 volumes with the same solvent mixture.

Uniformity of content. Complies with the test stated under Tablets.

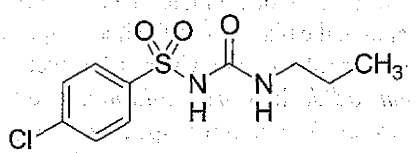
Powder one tablet, shake with 1 ml *of dilute hydrochloric acid* and 40 ml of *water* for 15 minutes, add sufficient *water* to produce 100.0 ml and mix. Centrifuge about 15 ml and to 10.0 ml of the clear, supernatant liquid add 2 ml of *1 M hydrochloric acid* and sufficient *water* to produce a solution containing about 0.0005 per cent w/v of Chlorpromazine Hydrochloride. Measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of $C_{17}H_{19}ClN_2S$,HCl in the tablet taking 915 as the specific absorbance at 254 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.1 g of Chlorpromazine Hydrochloride, add 5 ml of *dilute hydrochloric acid* and 200 ml of *water*. Shake for 15 minutes and add sufficient *water* to produce 500.0 ml. Centrifuge about 15 ml and to 5.0 ml of the clear, supernatant liquid add 10 ml of *dilute hydrochloric acid* and sufficient *water* to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7). Calculate the content of $C_{17}H_{19}ClN_2S$,HCl, taking 915 as the specific absorbance at 254 nm.

Storage. Store protected from light.

Chlorpropamide



$C_{10}H_{13}ClN_2O_3S$ Mol. Wt. 276.7

Chlorpropamide is 1-(4-chlorobenzenesulphonyl)-3-propylurea.

Chlorpropamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{13}ClN_2O_3S$, calculated on the dried basis.

Category. Hypoglycaemic.

Description. A white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorpropamide IPRS* or with the reference spectrum of chlorpropamide.

B. Dissolve 0.16 g in 50 ml of *methanol*, dilute 5 ml to 100 ml with *0.01 M hydrochloric acid* and dilute 5 ml of the solution to 100 ml with *0.01 M hydrochloric acid*. When examined in the range 220 nm to 360 nm, the resulting solution shows an absorption maximum at about 232 nm, about 0.48 (2.4.7).

C. Boil 0.1 g with 8 ml of 50 per cent w/v solution of *sulphuric acid* under a reflux condenser for 30 minutes, cool and filter, reserving the filtrate for test D. The precipitate, after recrystallisation from *water* and drying, melts at about 143° (2.4.21).

D. Make the filtrate reserved in test C alkaline with *sodium hydroxide solution* and heat; an ammonical odour is produced.

E. Heat 0.1 g with 1 g of *anhydrous sodium carbonate* at a dull red heat for 10 minutes. Cool, extract the residue with *water* and filter. Acidify the filtrate with *dilute nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *chloroform*, 50 volumes of *methanol*, 30 volumes of *cyclohexane* and 11.5 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.6 g of the substance under examination in 10 ml of *acetone*.

Reference solution (a). A 0.02 per cent w/v solution of *4-chlorobenzenesulphonamide* in *acetone*.

Reference solution (b). A 0.02 per cent w/v solution of *1,3-dipropylurea IPRS* in *acetone*.

Reference solution (c). A 0.02 per cent w/v solution of the substance under examination in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air, heat at 110° for 10 minutes,

place the plate, while hot, in a tank of chlorine gas prepared by adding *hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of *potassium iodide* in *starch solution;* avoid prolonged exposure to cold air. Any spots corresponding to 4-chlorobenzenesulphonamide and 1,3-dipropylurea in the chromatogram obtained with the test solution are not more intense than the spots in the chromato-gram obtained with reference solution (a) and (b) respectively. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

Heavy metals (2.3.13). 0.66 g complies with the limit test for heavy metals, Method B (30 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g and dissolve in 50 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution*. Add 25 ml of *water* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02767 g of $C_{10}H_{13}ClN_2O_3S$.

Chlorpropamide Tablets

Chlorpropamide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorpropamide, $C_{10}H_{13}ClN_2O_3S$.

Usual strengths. 100 mg; 250 mg.

Identification

Extract a quantity of the powdered tablets containing 1 g of Chlorpropamide with five quantities, each of 4 ml, of *acetone*, filter and carefully evaporate the filtrate to dryness on a water-bath. The residue complies with the following tests.

A. Boil 0.1 g with 8 ml of 50 per cent w/v solution of *sulphuric acid* under a reflux condenser for 30 minutes, cool and filter, reserving the filtrate for test B. The precipitate, after recrystallisation from *water* and drying, melts at about 143° (2.4.21).

B. Make the filtrate reserved in test A alkaline with *sodium hydroxide solution* and heat; an ammonical odour is produced.

C. Heat 0.1 g with 1 g of *anhydrous sodium carbonate* at a dull red heat for 10 minutes. Cool, extract the residue with *water* and filter. Acidify the filtrate with *dilute nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

Tests

Dissolution (2.5.2).

Apparatus. No. 2 (Paddle),
Medium. 900 ml of a 0.68 per cent w/v solution of *potassium dihydrogen phosphate* adjusted to pH 7.4 by the addition of *1 M sodium hydroxide*.
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid* to obtain a solution containing about 10 µg of chlorpropamide per ml. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of $C_{10}H_{13}ClN_2O_3S$ taking 598 as the specific absorbance at 232 nm.

Q. Not less than 75 per cent of the stated amount of $C_{10}H_{13}ClN_2O_3S$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *chloroform*, 50 volumes of *methanol*, 30 volumes of *cyclohexane* and 11.5 volumes of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 0.6 g of Chlorpropamide with 10 ml of *acetone* and filter.

Reference solution (a). A 0.02 per cent w/v solution of *4-chlorobenzenesulphonamide* in *acetone*.

Reference solution (b). A 0.02 per cent w/v solution of *1,3-dipropylurea IPRS* in *acetone*.

Reference solution (c). A 0.02 per cent w/v solution of the substance under examination in *acetone*.

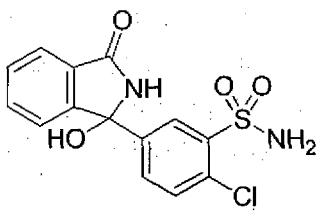
Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air, heat at 110° for 10 minutes, place the plate, while hot, in a tank of chlorine gas prepared by adding *hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of *potassium iodide* in *starch solution;* avoid prolonged exposure to cold air. Any spots corresponding to 4-chlorobenzenesulphonamide and 1,3-dipropylurea in the chromatogram obtained with the test solution are not more

intense than the spots in the chromatogram obtained with reference solution (a) and (b) respectively. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.25 g of Chlorpropamide and shake with 40 ml of *methanol* for 20 minutes, add sufficient *methanol* to produce 50.0 ml, mix, filter and dilute 5.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid*. Mix, dilute 10.0 ml of the solution to 250.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of $C_{10}H_{13}ClN_2O_3S$ taking 598 as the specific absorbance at 232 nm.

Chlorthalidone



$C_{14}H_{11}ClN_2O_4S$ Mol. Wt. 338.8

Chlorthalidone is (*RS*)-2-chloro-5-(1-hydroxy-3-oxoisoindolin-1-yl)benzenesulphonamide.

Chlorthalidone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{11}ClN_2O_4S$, calculated on the dried basis.

Category. Diuretic.

Description. A white to yellowish-white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorthalidone IPRS* or with the reference spectrum of chlorthalidone.

B. When examined in the range 230 nm to 360 nm, a 0.01 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 275 nm and at about 284 nm; absorbance at about 275 nm, about 0.6 and at about 284 nm, about 0.45 (2.4.7).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 197 volumes of *ethyl acetate* and 3 volumes of *water*.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *acetone*.

Reference solution. A 0.1 per cent w/v solution of *chlorthalidone IPRS* in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve 10 mg in 1 ml of *sulphuric acid*; an intense yellow colour is produced.

Tests

Appearance of solution. Dissolve 1.0 g in sufficient *2 M sodium hydroxide* to produce 10 ml. The solution is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solution (2.4.1).

Acidity. Dissolve 1 g in a mixture of 25 ml of *acetone* and 25 ml of *carbon dioxide-free water* with the aid of heat, cool and titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations is not more than 0.75 ml.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 2 volumes of a 0.2 per cent w/v solution of *sodium hydroxide*, 48 volumes of mobile phase B and 50 volumes of mobile phase A.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.0001 per cent w/v solution of *chlorthalidone IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica gel (5 µm),
- column temperature: 40°,
- mobile phase: A. a buffer solution prepared by dissolving 1.32 g of *diammonium hydrogen orthophosphate* in 900 ml of *water*, adjusted to pH 5.5 with *dilute orthophosphoric acid* and dilute to 1000 ml with *water*,

  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
16	65	35
21	50	50
35	50	50
45	65	35

Name	Relative retention time
Chlorthalidone impurity B[1]	0.7
Chlorthalidone impurity J[2]	0.9
Chlorthalidone(retention time: about 7 minutes)	1.0
Chlorthalidone impurity G[3]	6.0

[1]2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid,

[2]impurity of unknown structure with a relative retention of about 0.9,

[3](3*RS*)-3-(3,4-dichlorophenyl)-3-hydroxy-2,3-dihydro-1*H*-isoindol-1-one.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to impurity B is not more than 7 times the area of principal peak in the chromatogram obtained with the reference solution (0.7 per cent), the area of any peak due to impurity J is not more than 3 times the area of principal peak in the chromatogram obtained with the reference solution (0.3 per cent), the area of any peak due to impurity G is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 12 times the area of the principal peak in the chromatogram with the reference solution(1.2 per cent).

Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Chlorides (2.3.12). Triturate 0.5 g with 30 ml of *water*, shake for 5 minutes and filter. 15 ml of the filtrate complies with the limit test for chlorides. Use 5.0 ml of *chloride standard solution (25 ppm Cl)* to prepare the standard (500 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of *methanol*. Take 5.0 ml of the resulting solution, dilute with *water* to 50.0 ml and mix.

Reference solution. A 0.1 per cent w/v solution of *chlorthalidone IPRS* in *methanol*. Take 5.0 ml of the solution dilute with 50.0 ml of *water* and mix.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *0.01 M dibasic ammonium phosphate* and 40 volumes of *methanol* adjusted to pH 5.5 with *orthophosphoric acid*,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 25 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{11}ClN_2O_4S$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Chlorthalidone Tablets

Chlorthalidone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of chlorthalidone, $C_{14}H_{11}ClN_2O_4S$.

Usual strength. 50 mg.

Identification

Heat a quantity of the powdered tablets containing 0.2 g of Chlorthalidone with 20 ml of *acetone* on a water-bath for 10 minutes, cool and filter. Add 40 ml of *water* to the filtrate and heat on a water-bath for 20 minutes using a gentle current of air to remove the solvent. Cool to room temperature and allow to stand, filter and dry the crystals at 105° for 4 hours. The crystals comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorthalidone IPRS* or with the reference spectrum of chlorthalidone.

B. When examined in the range 230 nm to 360 nm, a 0.01 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 275 nm and at about 284 nm; absorbance at about 275 nm, about 0.6 and at about 284 nm, about 0.45 (2.4.7).

C. Wash with *water* a quantity of the crystals obtained in test A and dissolve 50 mg in 3 ml of *sulphuric acid*; an intense yellow colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 275 nm (2.4.7). Calculate the content of $C_{14}H_{11}ClN_2O_4S$ in the medium from the absorbance obtained from a solution of known concentration of *chlorthalidone IPRS*, prepared by dissolving in *methanol* and diluted with the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{14}H_{11}ClN_2O_4S$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *butanol* and 15 volumes of *1M ammonia*.

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Chlorthalidone with 5.0 ml of *acetone*, centrifuge and use the supernatant liquid.

Reference solution. A solution containing 0.01 per cent w/v of *2-(4-chloro-3-sulphamoylbenzoyl)benzoic acid IPRS* in *acetone*.

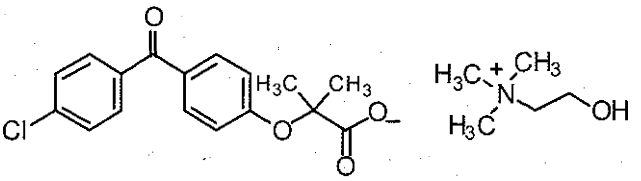
Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.1 g of Chlorthalidone, boil with 30 ml of *methanol* under a reflux condenser for 5 minutes, shake vigorously for 15 minutes, cool and filter; wash the residue with *methanol* and filter. Dilute the combined filtrate and washings to 100.0 ml with *methanol*. To 5.0 ml add 2 ml of *1M hydrochloric acid* and sufficient *methanol* to produce 50.0 ml. Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of $C_{14}H_{11}ClN_2O_4S$ taking 57.4 as the specific absorbance at 275 nm.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Choline Fenofibrate



$C_{22}H_{28}ClNO_5$ Mol Wt. 421.9

Choline Fenofibrate is 2-[4-(4-Chlorophenylcarbonyl) phenoxy]-2-methylpropanoate choline salt.

Choline Fenofibrate contains fenofibric acid not less than 72.0 per cent and not more than 76.0 per cent and choline not less than 23.0 per cent and not more than 26.0 per cent, calculated on the dried basis.

Category. Antihyperlipidaemic.

Description. A white to off white crystalline powder.

Identification

A. In the test for Related substances, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in mobile phase and dilute to 25.0 ml with mobile phase.

Reference solution. A 0.1 per cent w/v solution of *choline fenofibrate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *water* ,adjusted to pH 2.5 with *phosphoric acid* and 70 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 286 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.2 per cent and the sum of areas of all the secondary peaks is not more than 0.5 per cent, calcuated by area normalisation.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. *For Fenofibric acid*—Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in mobile phase and dilute to 25.0 ml with mobile phase. Dilute 5.0 ml of the solution to 20.0 ml with mobile phase.

Reference solution. A 0.025 per cent w/v solution of *fenofibric acid IPRS* in the mobile phase.

Use the chromatographic system described as in the Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of fenofibric acid.

For Choline — Weigh 0.3 g, dissolve in 70 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1ml of *0.1 M perchloric acid* is equivalent to 0.0104 g of choline.

Chorionic Gonadotrophin

Human Chorionic Gonadotrophin

Chorionic Gonadotrophin is a dry, sterile preparation of placental glycoproteins that has luteinising activity. It is extracted from the urine of pregnant women. The material is sterilised by filtration and dried under reduced pressure or freeze-dried.

Chorionic Gonadotrophin contains not less than 2500 Units per mg.

Category. Gonadotrophic hormone.

Description. A white or almost white, amorphous powder.

Identification

It causes an increase in the weight of the seminal vesicles or of the prostate glands of immature male rats when administered as directed in the Assay.

Tests

Appearance of solution. A 1.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

Water (2.3.43). Not more than 5.0 per cent, determined on 0.2 g.

Assay. Carry out the biological assay of chorionic gonadotrophin described below.

Standard preparation. The 3rd International Standard for Chorionic Gonadotrophin, human, established in 1986, consisting of a freeze-dried extract of human chorionic gonadotrophin with human albumin (supplied in ampoules containing 650 Units), or another suitable preparation the potency of which has been determined in relation to the International Standard.

Dissolve a sufficient quantity corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

Test preparation. Dissolve a sufficient quantity of the preparation under examination corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

Use immature male rats of the same strain, approximately 21 days old and of approximately equal weight within the range 25 to 35 g. Assign the rats at random to four equal groups of at least eight animals. If sets of four littermates are available, allot one littermate from each set at random to each group and mark according to the litter.

Choose two doses of the standard preparation and two of the test solution such that the smaller dose is sufficient to produce a positive response in some of the rats and the larger dose does not produce a maximum response in all of the rats. As an initial approximation, doses of 7.5 and 15 Units may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely.

Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, kill the rats and remove the seminal vesicles or the prostate glands from each animal. Remove any extraneous fluid and tissue from the vesicles or glands and weigh them immediately. Calculate the result of the assay by standard statistical methods using the weight of the vesicles or prostate glands as the response.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of the stated potency.

Chorionic Gonadotrophin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 15.0 Endotoxin Units per ml of a solution prepared in the following manner. Dissolve a quantity in *water BET* to obtain a solution containing 500 units of chorionic gonadotrophin per ml. Carry out the test using Maximum Valid dilution of the solution calculated from the declared sensitivity of the lysate used in the test.

Chorionic Gonadotrophin intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirements.

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity (2.2.1) using a quantity equivalent to 1000 Units dissolved in 0.5 ml of *sodium chloride injection* and observing the animals for 48 hours.

Storage. Store protected from light in a tamper-evident container, which is sealed so as to exclude micro-organisms, in a refrigerator (2° to 8°).

Labelling. The label states (1) the number of Units contained in the container; (2) the number of Units per mg; (3) whether or not it is intended for use in the manufacture of parenteral preparations.

Chorionic Gonadotrophin Injection

Chorionic Gonadotrophin Injection is a sterile material consisting of Chorionic Gonadotrophin with or without excipients such as buffers, diluents or other inert substances such as Lactose or Sodium Chloride. It may also contain an antimicrobial agent. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections or a suitable diluent supplied by the manufacturer, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Chorionic Gonadotrophin Injection contains not less than 80.0 per cent and not more than 125.0 per cent of the stated potency.

Usual strengths. 500, 1000, 2000, 5000 and 10,000 Units in each sealed container.

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Identification

It causes an increase in the weight of the seminal vesicles or of the prostate glands of immature male rats when administered as directed in the Assay.

Tests

pH (2.4.24). 6.0 to 8.0, determined in a 1.0 per cent w/v solution.

Assay. Carry out the biological assay of chorionic gonadotrophin described below.

Standard preparation. The 3rd International Standard for Chorionic Gonadotrophin, human, established in 1986, consisting of a freeze-dried extract of human chorionic gonadotrophin with human albumin (supplied in ampoules containing 650 Units), or another suitable preparation the potency of which has been determined in relation to the International Standard.

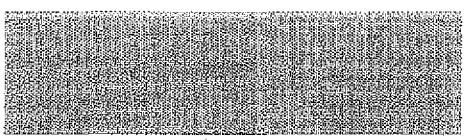
Dissolve a sufficient quantity corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

Test preparation. Dissolve a sufficient quantity of the injection under examination corresponding to the daily doses to be used in sufficient *albumin-phosphate buffer pH 7.2* so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

Use immature male rats of the same strain, approximately 21 days old and of approximately equal weight within the range 25 to 35 g. Assign the rats at random to four equal groups of at least eight animals. If sets of four littermates are available, allot one littermate from each set at random to each group and mark according to the litter.

Choose two doses of the standard preparation and two of the test solution such that the smaller dose is sufficient to produce a positive response in some of the rats and the larger dose does not produce a maximum response in all of the rats. As an initial approximation, doses of 7.5 and 15 Units may be tried although the dose will depend on the sensitivity of the animals used that may vary widely.

Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, kill the rats and remove the seminal vesicles or the prostate glands from each animal. Remove any extraneous fluid and tissue from the vesicles or glands and weigh them immediately.



Calculate the result of the assay by standard statistical methods using the weight of the vesicles or prostate glands as the response.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of the stated potency.

Bacterial endotoxins (2.2.3). Not more than 15 Endotoxin Units per ml of a solution prepared in the following manner. Dissolve the contents of a sealed container in *water BET* to obtain a solution containing 500 units of chorionic gonadotrophin per ml. Carry out the test using Maximum Valid Dilution of the solution calculated from the declared sensitivity of the lysate used in the test.

Abnormal toxicity (2.2.1). Use a quantity equivalent to 1000 Units dissolved in 0.5 ml of *sodium chloride injection* and observing the animals for 48 hours.

Storage. Store protected from light in containers, which are sealed so as to exclude micro-organisms, at a temperature not exceeding 20°.

Labelling. The label states (1) the number of Units contained in the sealed container; (2) the name(s) of any added substance(s).

Chymotrypsin

Chymotrypsin is a proteolytic enzyme obtained by the activation of chymotrypsinogen extracted from the pancreas of beef *(Bos taurus* L.*)*. It has an activity of not less than 5.0 microkatals per milligram. In solution it has maximal enzymic activity at about pH 8; the activity is reversibly inhibited at pH 3, the pH at which it is most stable.

Category. Proteolytic enzyme.

Production

The animals from which chymotrypsin is derived must fulfil the requirements for the health of animals suitable for human consumption. Furthermore, the tissues used shall not include any specified risk material as defined by any relevant international or, where appropriate, national legislation.

The method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Histamine (2.2.7). Not more than 1 µg (calculated as histamine base) per 5 microkatals of chymotrypsin activity. Before carrying out the test, heat the solution of the substance under examination on a water-bath for 30 minutes.

Description. A white or almost white, crystalline or amorphous powder.

Identification

Substrate solution. To 24 mg of *acetyltyrosine ethyl ester* add 0.2 ml of *ethanol (95 per cent)*, and swirl until solution is effected. Add 2.0 ml of *0.067 M phosphate buffer solution pH 7.0* and 1 ml of *methyl red mixed solution* and dilute to 10.0 ml with *water*.

A. A 1.0 per cent w/v solution in *carbon dioxide-free water* (Solution A). Dilute 1 ml of solution A to 10 ml with *water*. In a depression in a white spot plate, mix 0.05 ml of the solution with 0.2 ml of substrate solution; a purple colour develops.

B. Dilute 0.5 ml of solution A to 5 ml with *water*. Add 0.1 ml of a 2 per cent w/v solution of *tosylphenylalanylchloromethane* in *ethanol (95 per cent)*. Adjusted to pH 7.0 and shake for 2 hours. In a depression in a white spot plate, mix 0.05 ml of the solution with 0.2 ml of the substrate solution; no colour develops within 3 minutes of mixing.

Tests

Appearance of solution. Solution A is not more opalescent than Opalescence standard OS2 (2.4.1).

pH (2.4.24). 3.0 to 5.0, determined in solution A.

Light absorption. Dissolve 30 mg in 100.0 ml of *0.001 M hydrochloric acid*. The solution shows an absorption maximum at 281 nm and a minimum at 250 nm (2.4.7). The specific absorbance at the absorption maximum is 18.5 to 22.5 and at the absorption minimum is not more than 8.

Trypsin. Transfer to a depression in a white spot plate 0.05 ml of *tris (hydroxymethyl)aminomethane buffer solution pH 8.1* and 0.1 ml of solution A. Add 0.2 ml of substrate solution. At the same time and in the same manner, prepare a reference solution using the substance under examination to which not more than 1 per cent w/w of *trypsin* has been added. Start a timer. No colour appears in the test solution within 3-5 minutes after the addition of the substrate solution. A purple colour is produced in the control solution.

Substrate solution. To 98.5 mg of *tosylarginine methyl ester hydrochloride*, suitable for assaying trypsin, add 5 ml of *tris(hydroxymethyl)aminomethane buffer solution pH 8.1* and swirl to dissolve. Add 2.5 ml of *methyl red mixed solution* and dilute to 25.0 ml with *water*.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying at 60° at a pressure not exceeding 0.7 kPa for 2 hours.

Assay. The activity of chymotrypsin is determined by comparing the rate at which it hydrolyses *acetyltyrosine ethyl ester* with the rate at which *chymotrypsin IPRS* hydrolyses the same substrate under the same conditions.

Apparatus

Use a reaction vessel of about 30 ml capacity provided with:

– a device that will maintain a temperature of 25.0 ± 0.1°;
– a stirring device, for example a magnetic stirrer;
– a lid with holes for the insertion of electrodes, the tip of a burette, a tube for the admission of nitrogen and the introduction of reagents.

An automatic or manual titration apparatus may be used. For the latter the burette is graduated in 0.005 ml and the pH meter is provided with a wide scale and glass- calomel or glass-silver-silver chloride electrodes.

Test solution. Dissolve 25 mg of the substance under examination in 250.0 ml of *0.001 M hydrochloric acid.*

Reference solution. A 0.01 per cent w/v solution of *chymotrypsin IPRS* in *0.001 M hydrochloric acid.*

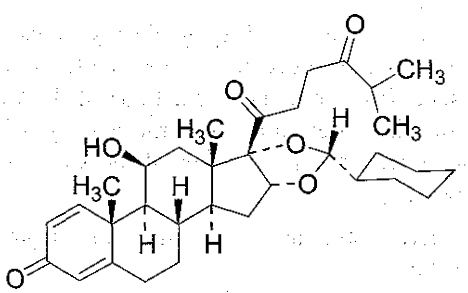
Store the solutions at below 5°. Warm 1 ml of each solution to about 25° over 15 minutes and use 50 µl of each solution (corresponding to about 25 nanokatals) for each titration. Carry out the titration in an atmosphere of nitrogen. Transfer 10.0 ml of *0.01 M calcium chloride solution* to the reaction vessel and, while stirring, add 0.35 ml of *0.2 M acetyltyrosine ethyl ester solution* . When the temperature is steady at 25.0 ± 0.1° (after about 5 minutes) adjusted to pH exactly 8.0 with *0.02 M sodium hydroxide*. Add 50 µl of the test solution (equivalent to about 5 µg of the substance under examination) and start a timer. Maintain the pH at 8.0 by the addition of *0.02 M sodium hydroxide*, noting the volume added every 30 seconds. Calculate the volume of *0.02 M sodium hydroxide* used per second between 30 seconds and 210 seconds. Carry out a titration in the same manner using the reference solution and calculate the volume of *0.02 M sodium hydroxide* used per second.

Calculate the activity in microkatals per milligram.

Storage. Store protected from light, in a refrigerator (2° to 8°).

Labelling. The quantity of chymotrypsin and the total activity in microkatals per container; for the amorphous substance, that it is hygroscopic.

Ciclesonide



$C_{32}H_{44}O_7$ Mol. Wt. 540.7

Ciclesonide is (11β,16α)-16,17-[(*R*)-cyclohexylmethylene] bis(oxy)-11-hydroxy-21-(2-methyl-1-oxopropoxy)pregna-1,4-diene-3,20-dione.

Ciclesonide contains not less than 98.0 per cent and not more than 102.0 per cent of ciclesonide, $C_{32}H_{44}O_7$, calculated on the anhydrous basis.

Category. Glucocorticoid.

Description. A white to-off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ciclesonide* RS or with the reference spectrum of ciclesonide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +90.0° to +98.0°, determined in a 0.5 per cent w/v solution in *methanol.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of *methanol.*

Reference solution. A 0.001 per cent w/v solution of *ciclesonide IPRS* in *methanol.*

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
– mobile phase: A. dilute 1 volume of *orthophosphoric acid* to 1000 ml with *water*,
B. *acetonitrile,*
– a gradient programme using the conditions given below,
– flow rate: 1.5 ml per minute,
– spectrophotometer set at 240 nm,
– injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
20	25	75
40	25	75
45	65	35
55	65	35

Inject the reference solution. The test is not valid unless the column efficiency is not less than 30000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities found is not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of *methanol*. Dilute 5 ml of the resulting solution to 50.0 ml with *methanol*.

Reference solution. A 0.01 per cent w/v solution of *ciclesonide IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of 0.1 per cent *orthophosphoric acid* and 70 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{32}H_{44}O_7$.

Storage. Store protected from light, at a temperature not exceeding 30°.

Ciclesonide Inhalation

Ciclesonide Inhalation is a suspension or solution of microfine Ciclesonide in a suitable liquid filled in a suitable pressurized container. It may contain suitable pharmaceutical aids such as surfactants, stabilizing agents.

Ciclesonide Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of ciclesonide, $C_{32}H_{44}O_7$, per inhalation by actuation of the valve.

Usual strengths. 80 µg per metered dose; 160 µg per metered dose.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Other tests. Comply with the tests stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of equal volumes of *water* and *acetonitrile*.

Test solution. Prepare using the solvent mixture as described under the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Reference solution (a). A 0.04 per cent w/v solution of *ciclesonide IPRS* in *acetonitrile*.

Reference solution (b). Dilute reference solution (a) with the solvent mixture to obtain a solution containing 32 µg of Ciclesonide per ml.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by diluting 1 ml of *orthophosphoric acid* to 1000 ml with *water* and 70 volumes of *acetonitrile*,
- flow rate: 3 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 200 µl or 100 µl.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 3500 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

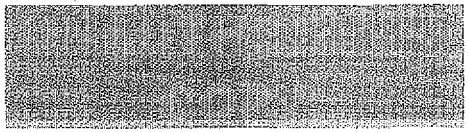
Inject reference solution (b) and the test solution.

Calculate the content of $C_{32}H_{44}O_7$ in the solution and the amount of $C_{32}H_{44}O_7$ delivered per actuation of the valve.

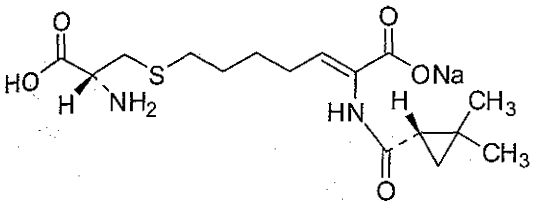
Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of $C_{32}H_{44}O_7$ delivered per actuation of the valve meets the requirements.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the amount of active ingredient delivered per inhalation.



Cilastatin Sodium



$C_{16}H_{25}N_2NaO_5S$ Mol. Wt. 380.4

Cilastatin sodium is sodium (*Z*)-7-[[(*R*)-2-amino-2-carboxyethyl]sulphanyl]-2-[[[(1*S*)-2,2-dimethyl-cyclopropyl]carbonyl]amino]hept-2-enoate.

Cilastatin Sodium contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{16}H_{25}N_2NaO_5S$, calculated on the anhydrous and solvent free basis.

Category. Antibacterial.

Description. A white or light yellow amorphous powder, hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cilastatin sodium IPRS* or with the reference spectrum of cilastatin sodium.

B. It gives reaction A of sodium (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *carbon-dioxide free water* (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 6.5 to 7.5, determined in solution A.

Specific optical rotation (2.4.22). +41.5° to +44.5°, determined in 1.0 per cent w/v solution in a mixture of 1 volume of *hydrochloric acid* and 120 volumes of *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 32 mg of the substance under examination in 20.0 ml of *water*.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with *water*. Dilute 5.0 ml of the solution to 100.0 ml with *water*.

Reference solution (b). Dilute 5.0 ml the test solution to 100.0 ml with *water*. Dilute 5.0 ml of the solution to 50.0 ml with *water*.

Reference solution (c). Dissolve 16 mg of the substance under examination in *dilute hydrogen peroxide solution* and dilute to 10.0 ml with the same solvent. Allow to stand for 30 minutes. Dilute 1 ml of the solution to 100 ml with *water*.

Reference solution (d). Dissolve 32 mg of *mesityl oxide* in 100 ml of *water*. Dilute 1 ml of the solution to 50 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: A. a mixture of 30 volumes of *acetonitrile* and 70 volumes of a 0.1 per cent v/v solution of *orthophosphoric acid* in *water*,
  B. a 0.1 per cent v/v solution of *orthophosphoric acid* in *water*,
- a gradient programme using the conditions given below,
- flow rate: 2.0 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	15	85
30	100	0
46	100	0
56	15	85

Equilibrate the column with a mixture of 15 per cent v/v of mobile phase A and 85 per cent v/v of mobile phase B. Inject separately each solution. Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 15 per cent of the full scale of the recorder.

Inject reference solution (a) and (c). The test is not valid unless, the chromatogram obtained with reference solution (c) shows three principal peaks: the first two peaks (cilastatin impurity A) may elute without being completely resolved and the capacity factor of the third peak (cilastatin) is not less than 10; in the chromatogram obtained with reference solution (a), the principal peak has a signal-to-noise ratio is not less than 5.0.

Inject reference solution (a), (b), (d) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (a) and any peak corresponding to the principal peak in the chromatogram obtained with reference solution (d).

Mesityl oxide, acetone and methanol. Not more than 1.0 per cent w/w of *acetone*, 0.5 per cent w/w of *methanol* and 0.4 per cent w/w of *mesityl oxide*.

Determine by gas chromatography (2.4.13).

Internal standard solution. Dissolve 0.5 ml of *propanol* in *water* and dilute to 1000 ml with the same solvent.

Test solution. Dissolve 0.2 g of the substance under examination in *water*, add 2.0 ml of the internal standard solution and dilute to 10.0 ml with *water*.

Reference solution. Dissolve 2.0 ml of *acetone*, 0.5 ml of *methanol* and 0.5 ml of *mesityl oxide* in *water* and dilute to 1000 ml with the same solvent. To 2.0 ml of the solution add 2.0 ml of the internal standard solution and dilute to 10.0 ml with *water*. This solution contains 316 µg of acetone, 79 µg of methanol and 86 µg of mesityl oxide per milliliter.

Chromatographic system
- a fused-silica column 30 m x 0.53 mm, packed with macrogol 20000 (film thickness 1.0 µm),
- temperature:
  column 50° from 0 to 2.5 minutes, 50° - 70° from 2.5 to 5 minutes and hold at 70° from 5 to 5.5 minutes,
  inlet port at 160°and detector at 220°,
- a flame-ionisation detector,
- flow rate: 9 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of the reference solution and the test solution. Calculate the contents of acetone, methanol and mesityl oxide.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Assay. Dissolve 0.3 g in 30 ml of *methanol* and add 5 ml of *water*. Add *0.1 M hydrochloric acid* to a pH of about 3.0. Titrate with *0.1 M sodium hydroxide*, determining the end point potentiometrically (2.4.25). Three jumps of potential are observed, titrate to the third equivalence point. Use the volume added between the first and third point of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01902 g of $C_{16}H_{25}N_2NaO_5S$.

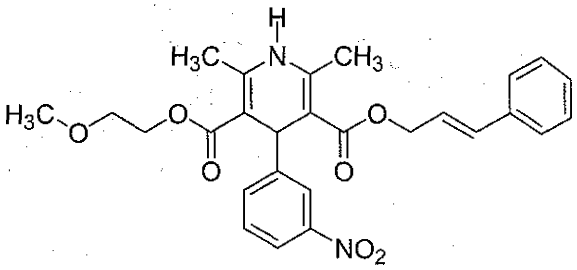
Cilastatin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.17 Endotoxin Units per mg of cilastatin sodium.

Storage. Store protected from moisture, at a temperature not exceeding 8°. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

Labelling. The label states, where applicable, that the substance is free from bacterial endotoxins.

Cilnidipine



$C_{27}H_{28}N_2O_7$ Mol. Wt. 492.5

Cilnidipine is 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 2-methoxyethyl (2*E*)-3-phenyl-2-propenyl ester.

Cilnidipine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{27}H_{28}N_2O_7$, calculated on the dried basis.

Category. Antihypertensive, calcium channel blocker.

Description. Light yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with the *cilnidipine IPRS* or with the reference spectrum of cilnidipine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Melting range (2.4.21). 108° to 113°.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution. A 0.0001 per cent w/v solution of *cilnidipine impurity A IPRS [bis(2-methoxyethyl)2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate]* and *cilnidipine IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Inertsil-ODS-3V),
- mobile phase: a mixture of 20 volumes of *water*, 40 volumes of *acetonitrile* and 40 volumes of *methanol*,

- flow rate: 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 20 µl.

The retention time of the cilnidipine impurity A is about 3.5 minutes and cilnidipine is about 7 minutes.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to cilnidipine impurity A and cilnidipine is not less than 6.5, the column efficiency for the peaks due to cilnidipine impurity A and cilnidipine is not less than 3000 theoretical plates and the tailing factor for the peaks due to cilnidipine impurity A and cilnidipine is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 3 times the retention time of the principal peak of cilnidipine (about 25 minutes). In the chromatogram obtained with the test solution, the area of secondary peak corresponding to cilnidipine impurity A is not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak of cilnidipine in the chromatogram obtained with reference solution (0.10 per cent) and the sum of the areas of all other secondary peaks is not more than 3 times the area of the principal peak of cilnidipine in the chromatogram obtained with the reference solution (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak of cilnidipine in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 60° for 1 hour under vacuum (reduce pressure of minimum 100 mm Hg).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50.0 mg of the substance under examination in *methanol* and dilute to 50.0 ml with *methanol*; further dilute 1.0 ml of the solution to 100.0 ml with *methanol*.

Reference solution. A 0.001 per cent w/v solution of *cilnidipine IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Prodigy ODS 3V),
- mobile phase: a mixture of 70 volumes of *acetonitrile* and 30 volumes of *0.01M sodium acetate buffer* prepared by dissolving 0.82 g of *sodium acetate anhydrous* in *water* and dilute to 1000 ml with *water* and adjusted to pH 5.0 with *dilute orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{28}N_2O_7$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Cilnidipine Tablets

Cilnidipine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Cilnidipine, $C_{27}H_{28}N_2O_7$.

Usual Strengths. 5 mg; 10 mg; 20 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 1.0 per cent w/v solution of *sodium lauryl sulphate* in *citro-phosphate* buffer solution prepared by dissolving 4.1363 g of *disodium hydrogen phosphate* and 0.475 g of *citric acid monohydrate* in 200 ml *water*, add 0.125 ml of *orthophosphoric acid* and dilute with *water* to 1000 ml. Adjusted to pH 6.8 with *2 M sodium hydroxide* or *ortho phosphoric acid* as required,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of medium and filter. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with dissolution medium. Measure the absorbance of the filtered solution at the maximum at about 243 nm (2.4.7). Calculate the content of *cilnidipine*, $C_{27}H_{28}N_2O_7$ in the medium from the absorbance obtained from a solution of known concentration of *cilnidipine IPRS* prepared by dissolving weighed quantity of *cilnidipine IPRS* in *methanol* and diluting further with the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{27}H_{28}N_2O_7$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the powdered tablets containing 50 mg of cilnidipine, disperse in 20 ml *methanol* with the aid of ultrasound for 20 minutes with intermediate shaking and dilute to 50.0 ml with mobile phase A, centrifuge and filter.

Reference solution. A 0.0002 per cent w/v solution of *cilnidipine IPRS* prepared by dissolving weighed quantity of *cilnidipine IPRS* completely in *methanol* and diluting further with the mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm), (Such as Kinetex C18),
- mobile phase: A. a mixture of 35 volumes of a buffer solution prepared by dissolving 2.0 g of *ammonium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *dilute ortho phosphoric acid* and 65 volumes of *methanol*,

  B. a mixture of 40 volumes of a buffer solution prepared by dissolving 2.0 g of *ammonium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *dilute phosphoric acid* and 60 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	45	55
12	45	55
20	35	65
28	35	65
45	35	65
55	45	55
60	45	55

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with test solution, the area of any secondary peak is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak of cilnidipine in the chromatogram obtained with reference solution (1.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in 50 ml solvent mixture with the aid of ultrasound for 15 minutes with intermediate shaking and dilute to volume to obtain a solution containing 0.005 per cent w/v of cilnidipine in solvent mixture.

Calculate the content of $C_{27}H_{28}N_2O_7$ in the Tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile,* 40 volumes of *methanol* and 20 volumes of a buffer solution prepared by dissolving 2.0 g of *ammonium dihydrogen phosphate,* in 1000 ml of *water*, adjusted to pH 3.0 with 10 per cent w/v solution of *ortho phosphoric acid.*

Test Solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 100 mg of cilnidipine, disperse in 50 ml solvent mixture with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with solvent mixture and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *cilnidipine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as YMC ODS-AM),
- mobile phase: a mixture of 25 volumes of a buffer solution prepared by dissolving 2.0 g of *ammonium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *dilute ortho phosphoric acid* and 75 volumes of *methanol*,
- column temperature: 40°,
- flow rate: 1 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 10 µl.

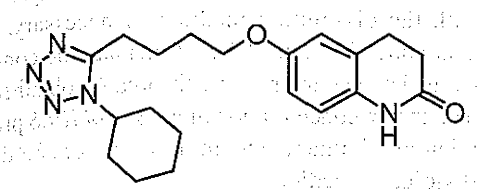
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{28}N_2O_7$ in the tablets.

Storage. Store protected from light and moisture.

Cilostazol



$C_{20}H_{27}N_5O_2$ Mol. Wt. 369.5

Cilostazol is 6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydroquinolin-2(1*H*)-one.

Cilostazol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{27}N_5O_2$, calculated on the dried basis.

Category. Indicated in intermittent claudication

Description. A white to off-white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cilostazol IPRS* or with the reference spectrum of cilostazol.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water* and 40 volumes of *acetonitrile*.

Test solution. Dissolve 20 mg of Cilostazol in 20 ml of *acetonitrile* and dilute to 50.0 ml with *water*.

Reference solution. A solution containing 0.05 per cent w/v each of *cilostazol IPRS* and *cilostazol impurity C IPRS* in *acetonitrile*. Dilute 4.0 ml of the solution to a 10.0 ml with *water*. Further dilute the solution to obtain a 0.00004 per cent w/v of cilostazol with the solvent mixture.

Use chromatographic system as described under Assay.

Name	Relative retention time	Correction factor
Cilostazol impurity A[1]	0.2	0.59
Cilostazol impurity B[2]	0.9	1.72
Cilostazol	1.0	1.0
Cilostazol impurity C[3]	1.9	—
Any other impurity	—	1.0

[1]6-hydroxy-3,4-dihydro-1*H*-quinolin-2-one,

[2]6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)-butoxy]-1*H*-quinolin-2-one,

[3]1-(4-(5-cyclohexyl-1*H*-tetrazol-1-yl)butyl)-6-(4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent). The sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (0.4 per cent).

Chlorides. Not more than 0.018 per cent.

Test solution. Dissolve 0.5 g of Cilostazol in 40 ml of *dimethylformamide*, add 6 ml of *dilute nitric acid* and dilute to 50 ml with *dimethylformamide*.

Reference solution. To 0.25 ml of *0.01 M hydrochloric acid*, add 6 ml of *dilute nitric acid* and dilute to 50 ml with *dimethylformamide*.

Add 1 ml of 5.0 per cent w/v solution of *silver nitrate* to the solutions, mix well and allow to stand for 5 minutes, protecting from direct sunlight. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely. The opalescence developed in the test solution is not more than that of the reference solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.3 per cent, determined on 1.0 g by drying in an oven at 110° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water* and 40 volumes of *acetonitrile*.

Test solution. Dissolve 20 mg of Cilostazol dissolve in 20 ml of *acetonitrile* and dilute to 50.0 ml with *water*. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.005 per cent w/v, each of, *cilostazol IPRS*, *cilostazol impurity A IPRS* and *cilostazol impurity B IPRS* in the solvent mixture.

Reference solution (b). A 0.1 per cent w/v solution of *cilostazol IPRS* in *acetonitrile*. Dilute 4.0 ml of the solution to a 10.0 ml with *water*. Further dilute the solution to obtain a solution of 0.004 per cent w/v of cilostazol with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3.5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 70 volumes of *water* and 30 volumes of *acetonitrile*,

B. a mixture of 50 volumes of *water* and 50 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent v/v)
0	100	0
6.5	50	50
10	0	100
20	0	100
20.1	100	0
28	100	0

Inject reference solution (a). The test is not valid unless the the resolution between cilostazol impuirity B and cilostazol is not less than 3.0, the tailing factor for cilostazol peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{20}H_{27}N_5O_2$.

Storage. Store protected from moisture and at room temperature.

Cilostazol Tablets

Cilostazol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cilostazol, $C_{20}H_{27}N_5O_2$.

Usual strengths. 50 mg; 100 mg; 200 mg.

Identification

A. To powdered tablets containing 0.1 g of Cilostazol, add 1 ml of *chloroform*, shake for 1 minute and filter. On the filtrate, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cilostazol IPRS* or with the reference spectrum of cilostazol.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 0.3 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution immediately, suitably diluted with the dissolution medium, if necessary, at the maximum at about 257 nm (2.4.7). Calculate the content of $C_{20}H_{27}N_5O_2$ in the medium from the absorbance obtained from a solution of known concentration of *cilostazol IPRS* prepared by dissolving in minimum amount of *methanol* and diluting with the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{27}N_5O_2$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. A 0.004 per cent w/v solution of *benzophenone* in *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of Cilostazol in the internal standard solution and dilute with the same solution to obtain a solution containing 0.01 per cent w/v of cilostazol and filter.

Reference solution. A 0.01 per cent w/v solution of *cilostazol IPRS* in the internal standard solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *acetonitrile*, 30 volumes of *methanol* and 100 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

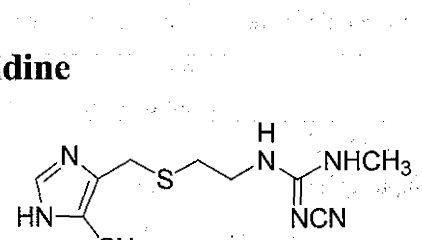
Inject the reference solution. The test is not valid unless the resolution between the cilostazol and benzophenone peak is not less than 9.0 and the relative standard deviation for replicate injections of the principal peakis not more than 1.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{27}N_5O_2$ in the tablets.

Storage. Store protected from light and moisture.

Cimetidine



$C_{10}H_{16}N_6S$. Mol Wt. 252.3

Cimetidine is 2-cyano-1-methyl-3-[2-(5-methylimidazol-4-ylmethylthio)ethyl]guanidine.

Cimetidine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{10}H_{16}N_6S$, calculated on the dried basis.

Category. Antiulcer.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6), using a *potassium bromide* dispersion obtained from the solid state without prior solvent treatment. Compare the spectrum with that obtained with *cimetidine IPRS* or with the reference spectrum of cimetidine. No shoulder or peak should be discernible at 1190 cm^{-1}.

B. When examined in the range 210 nm to 360 nm, a 0.0008 per cent w/v solution in *1 M sulphuric acid* shows an absorption maximum at 218 nm and a minimum at 260 nm (2.4.7).

C. In the test for related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

D. Dissolve about 1 mg in a mixture of 1 ml of *ethanol* and 5 ml of a freshly prepared 2 per cent w/v solution of *citric acid* in *acetic anhydride*. Heat in a water-bath for 10 to 15 minutes; a reddish violet colour is produced.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a). A 0.004 per cent w/v solution of *cimetidine IPRS* in mobile phase A.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 0.4 volume of *diethylamine* and 780 volumes of a 0.11 per cent w/v solution of *sodium hexanesulphonate*, adjusted to pH 2.8 with *orthophosphoric acid* and 250 volumes of *methanol*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
60	100	0
65	90	10
120	90	10

Name	Relative retention time	Correction factor
Cimetidine impurity G[1]	0.2	0.6
Cimetidine impurity E[2]	0.4	0.7
Cimetidine (Retention time: about 18 minutes)	1.0	—
Cimetidine impurity D[3]	1.5	3.3
Cimetidine impurity C[4]	1.6	2.5
Cimetidine impurity B[5]	2.0	—
Cimetidine impurity H[6]	2.3	—
Cimetidine impurity F[7]	4.6	—

[1]2-cyano-1,3-dimethylguanidine,

[2]2-cyano-1-methyl-3[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl] sulfinyl]ethyl] guanidine,

[3]1-methyl-3-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]sulfanyl]ethyl] guanidine,

[4]1-[(methylamino)[[2-[[(5-methyl-1*H*- imidazol-4-yl)methyl]sulfanyl] ethyl]amino] methylidene]urea,

[5]methyl 3-cyano-1-[2-[[(5-methyl-1*H*-imidazol-4- yl)methyl]sulfanyl] ethyl]carbamimidate,

[6]1,1'-(disulfanediyldiethylene)bis(2-cyano-3-methylguanidine),

[7]2-cyano-1,3-bis[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]sulfanyl] ethyl]guanidine.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g and dissolve in 75 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02523 g of $C_{10}H_{16}N_6S$.

Storage. Store protected from light.

Cimetidine Tablets

Cimetidine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cimetidine, $C_{10}H_{16}N_6S$.

Usual strengths. 200 mg; 400 mg; 800 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Cimetidine with 10 ml of *methanol*, filter, evaporate the filtrate to dryness using gentle heat and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cimetidine IPRS* or with the reference spectrum of cimetidine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (d).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary with dissolution medium at 218 nm. Calculate the content of cimetidine, $C_{10}H_{16}N_6S$ in the medium from the absorbance obtained from a solution of known concentration of *cimetidiene IPRS* in the same medium.

Q. Not less than 80 per cent of the stated amount of $C_{10}H_{16}N_6S$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase (a). A mixture of 65 volumes of *ethyl acetate*, 20 volumes of *methanol* and 15 volumes of *strong ammonia solution*.

Mobile phase (b). A mixture of 84 volumes of *ethyl acetate*, 8 volumes of *methanol* and 8 volumes of *strong ammonia solution*.

Test solution (a). Add 20 ml of *methanol* to a quantity of the powdered tablets containing 1 g of Cimetidine, mix with the aid of ultrasound for 2 minutes, shake for 3 minutes and filter using a suitable 0.2 µm filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of test solution (b) to 20.0 ml with *methanol*.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with *methanol*. Dilute 20.0 ml of the solution to 100.0 ml with *methanol*.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 10.0 ml with *methanol*.

Reference solution (d). Dissolve 10 mg of *cimetidine IPRS* in 2 ml of *methanol*.

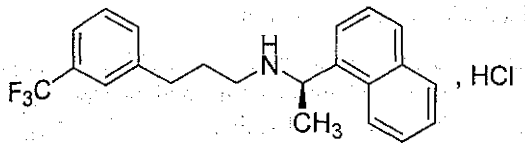
Apply separately to two plates 4 µl of each solution. Allow the first plate to stand for 15 minutes in the tank saturated with vapour from mobile phase (a). Develop the second plate using mobile phase (b). After development, dry the plates in a current of air, expose to iodine vapour until maximum contrast of the spots has been obtained and examine under ultraviolet light at 254 nm. The following limits apply to both methods. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows a clearly visible spot.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.25 g of Cimetidine and stir with 20 ml of warm *methanol*. Filter and repeat the extraction with three quantities, each of 20 ml, of warm *methanol*. Evaporate the combined filtrate and washings to dryness and dissolve the residue in 75 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02523 g of $C_{10}H_{16}N_6S$.

Cinacalcet Hydrochloride



$C_{22}H_{22}F_3N,HCl$ Mol. Wt. 393.9

Cinacalcet Hydrochloride is (*R*)-*N*-(3-(3-(trifluoromethyl) phenyl)propyl)-1-(1-napthyl)ethylamine hydrochloride.

Cinacalcet Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{22}F_3N,HCl$, calculated on the dried basis.

Category. Antihyperparathyroid.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cinacalcet hydrochloride IPRS* or with the reference spectrum of cinacalcet hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction A of chlorides (2.3.1)

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of equal volumes of a buffer solution containing *0.01M sodium perchlorate*, adjusted to pH 2.5 with *perchloric acid* and *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution. A 0.0005 per cent solution of *cinacalcet hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with phenyl group (3.5 µm) (Such as Zorbax SB-Phenyl),
- column temperature: 60°,
- mobile phase: A. *0.01M sodium perchlorate*, adjusted to pH 2.5 with *perchloric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 5µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
3	60	40
16	40	60
20	40	60
21	60	40
26	60	40

Name	Relative retention time	Correction factor
Cinacalcet impurity A[1]	0.22	0.44
Cinacalcet impurity B[2]	0.94	6.67
Cinacalcet (Retention time: about 7 minutes)	1.0	—
Cinacalcet impurity C[3]	1.32	1.16

[1]1-(Naphthalen-1-yl) ethanamine,

[2]Methanesulphonic acid 3-(3- trifluoro methyl-phenyl)-propyl ester,

[3]1-Naphthalen-1-yl-ethyl)-[3-(3-trifluoromethyl-cyclohexyl)-propyl]-amine.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0.

Inject the reference solution and test solution. In the chromatogram obtained with the test solution the area of each peak due to cinacalcet impurity A, cinacalcet impurity B and cinacalcet impurity C is not more than 0.3 times the area of principal peak in the chromatogram obtained with the reference solution (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than two times the area of the principal peak in the chromatogram with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Related substances.

Solvent mixture. A mixture of equal volumes of a buffer solution containing *0.01M sodium perchlorate*, adjusted to pH 2.5 with *perchloric acid* and *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *cinacalcet hydrochloride IPRS* in solvent mixture.

Chromatographic system
- column temperature: 40°,
- mobile phase: a mixture of 55 volumes of a buffer solution containing *0.01 M sodium perchlorate*, adjusted to pH 2.5 with *perchloric acid* and 45 volumes of *acetonitrile*,
- injection volume: 5 µl.

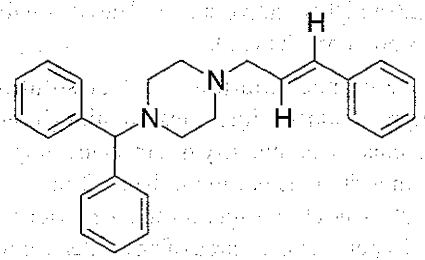
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{22}F_3N,HCl$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Cinnarizine



$C_{26}H_{28}N_2$ Mol. Wt. 368.5

Cinnarizine is (*E*)-1-(diphenylmethyl)-4-(3-phenylprop-2-enyl)piperazine.

Cinnarizine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{26}H_{28}N_2$, calculated on the dried basis.

Category. Antihistaminic.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cinnarizine IPRS* or with the reference spectrum of cinnarizine.

B. In the test for Related substances the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak due to cinnarizine in the chromatogram obtained with reference solution (a).

C. Dissolve 0.2 g of *anhydrous citric acid* in 10 ml of *acetic anhydride* in a water-bath at 80° and maintain the temperature of the water-bath at 80° for 10 minutes. Add about 20 mg of the substance under examination; a purple colour is produced.

Tests

Appearance of solution. A 2.5 per cent w/v solution in *dichloromethane* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity or Alkalinity. Suspend 0.5 g in 15 ml of *water*. Boil for 2 minutes, cool and filter. Dilute the filtrate to 20 ml with *carbon dioxide-free water*. To 10 ml add 0.1 ml of *phenolphthalein solution* and 0.25 ml of *0.01 M sodium hydroxide*; the solution is pink. To 10 ml add 0.1 ml of *methyl red solution* and 0.25 ml of *0.01 M hydrochloric acid*; the solution is red.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 10.0 ml of the *methanol*.

Reference solution (a). Dissolve 12.5 mg of *cinnarizine IPRS* and 15.0 mg of *flunarizine dihydrochloride IPRS* in 100.0 ml of the *methanol*. Dilute 1.0 ml of the solution to 20.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 20.0 ml with the same solvent.

Chromatographic system
- a stainless steel column 10 cm x 4 mm packed with base-deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. 1 per cent w/v solution of *ammonium acetate*,
  B. 0.2 per cent v/v solution of *glacial acetic acid* in *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
20	10	90
25	10	90
28	75	25

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to cinnarizine and flunarizine is not less than 5.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent), the sum of area of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). Dissolve 1.0 g in a mixture of 85 volumes of *acetone* and 15 volumes of *water* and add *dilute hydrochloric acid* until dissolution is complete. Dilute to 20 ml with the same mixture of *acetone* and *water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb)* obtained by diluting *lead standard solution (100 ppm Pb)* with the mixture of *acetone* and *water*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Weigh accurately about 0.15 g and dissolve in a mixture of 70 volumes of *2-butanone* and 10 volumes of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *α-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01843 g of $C_{26}H_{28}N_2$.

Cinnarizine Tablets

Cinnarizine tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cinnarizine, $C_{26}H_{28}N_2$.

Usual strength. 25 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.1 g of Cinnarizine with 20 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cinnarizine IPRS* or with the reference spectrum of cinnarizine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of gastric juice, artificial (without enzyme) prepared by dissolving 2.0 g of *sodium chloride* in 80 ml of *1M hydrochloric acid* and dilute to 1000 ml with *water*,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 253 nm (2.4.7). Calculate the content of $C_{26}H_{28}N_2$ in the medium from the absorbance obtained from a solution of known concentration of *cinnarizine IPRS*.

Q. Not less than 70 per cent of the stated amount of $C_{26}H_{28}N_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a suitable quantity of the powdered tablets containing 25 mg of Cinnarizine with *methanol*, dilute to 10 ml with the same solvent and filter.

Reference solution (a). Dissolve 12.5 mg of *cinnarizine IPRS* and 15 mg of *flunarizine dihydrochloride IPRS* in *methanol* and dilute to 100 ml with the same solvent. Dilute 1 ml of the solution to 20 ml with *methanol*.

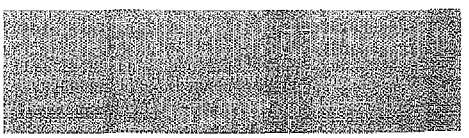
Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 20.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 10 cm x 4 mm, packed with base-deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a 1.0 per cent w/v solution of *ammonium acetate*,
  B. a 0.2 per cent v/v solution of *glacial acetic acid* in *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Time (min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
20	10	90
25	10	90
30	75	25

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the



chromatogram obtained is at least 50 per cent of the full scale of the recorder. If necessary, adjust the concentration of *glacial acetic acid* in mobile phase B to obtain a horizontal base-line in the chromatogram.

Inject reference solution (a). When the chromatogram is recorded in the prescribed conditions, the retention times are: cinnarizine about 11 min and flunarizine about 11.5 minutes. The test is not valid unless the resolution between the peaks corresponding to cinnarizine and flunarizine is at least 5.0. If necessary, adjust the time programme for the gradient elution.

Inject the blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution: the area of any peak, other than the principal peak, is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of the peaks, other than the principal peak, is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak due to the blank and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under tablets.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 25 mg of Cinnarazine with *methanol*, dilute to 50.0 ml with the same solvent and filter. Dilute 5.0 ml of the solution to 50.0 ml with *methanol*.

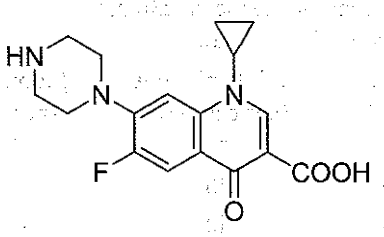
Reference solution. A 0.005 per cent w/v solution of *cinnarzine IPRS* in *methanol*.

Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{28}N_2$ in the tablets.

Storage. Store protected from light.

Ciprofloxacin



$C_{17}H_{18}FN_3O_3$ Mol. Wt. 331.4

Ciprofloxacin is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid.

Ciprofloxacin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{18}FN_3O_3$, calculated on the dried basis.

Category. Antibacterial.

Description. A white to pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained from *ciprofloxacin IPRS* or with the reference spectrum of ciprofloxacin.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *acetonitrile*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *6 M ammonia*.

Reference solution. A 1 per cent w/v solution of *ciprofloxacin IPRS* in *6 M ammonia*.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine under ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 2.5 per cent w/v solution in *0.1 M hydrochloric acid* is clear (2.4.1).

Related substances. Carry out the method described under Assay and calculate the percentage of each impurity from the chromatogram obtained with the test solution. The content of ciprofloxacin ethylenediamine analog or of any other individual impurity peak found is not more than 0.2 per cent and the sum of all the impurity peaks is not more than 0.5 per cent.

Fluoroquinolonic acid. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia* solution and 10 volumes of *acetonitrile*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *0.1 M acetic acid*.

Reference solution. Weigh 10 mg of *fluoroquinolonic acid IPRS*, add 0.1 ml of *6 M ammonia* and dilute to 100.0 ml with *water*. Dilute 2.0 ml of the solution to 10.0 ml with *water*.

Apply to the plate 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes. Remove the plate and place it in a chamber containing the mobile phase. After development, dry the plate in air for 15 minutes and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the spot of fluoroquinolonic acid is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides (2.3.12). To 2.0 g add 30 ml of *water*, shake for 5 minutes and filter through a chloride-free filter paper. 15 ml of the filtrate complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). Dissolve 0.75 g in 5.0 ml of *2 M acetic acid* and 20.0 ml of *water*. 10 ml of the resulting solution complies with the limit test for sulphates (400 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 120° for 6 hours at a pressure not exceeding 0.7 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 25 mg, add 0.2 ml of a solution containing 7 per cent v/v of *orthophosphoric acid* and add sufficient of the mobile phase to produce 50.0 ml.

Reference solution (a). Prepare in the same manner as the test solution using an accurately weighed quantity of *ciprofloxacin IPRS* in place of the substance under examination.

Reference solution (b). A 0.05 per cent w/v solution of *ciprofloxacin ethylenediamine analog IPRS* in reference solution (a).

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperaure: 30° ± 1°,
- mobile phase: a mixture of 87 volumes of *0.025 M orthophosphoric acid*, previously adjusted to pH 3.0 ± 0.1 with *triethylamine* and 13 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume: 10 µl.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6.

Inject reference solution (a). The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{18}FN_3O_3$.

Ciprofloxacin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per mg of ciprofloxacin.

Storage. Store protected from light.

Ciprofloxacin Injection

Ciprofloxacin Injection is a sterile solution of Ciprofloxacin or Ciprofloxacin Hydrochloride in 5 per cent Dextrose Injection or in Sodium Chloride Injection prepared with the aid of Lactic Acid.

Ciprofloxacin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ciprofloxacin, $C_{17}H_{18}FN_3O_3$.

Usual strength. 2 mg per ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber.

Mobile phase. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *acetonitrile*.

Test solution. Dilute sufficient of the injection with *water* to obtain a solution containing the equivalent of 0.05 per cent w/v of Ciprofloxacin.

Reference solution. A 0.05 per cent w/v solution of *ciprofloxacin IPRS* in *6 M ammonia*.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes

and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine under ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.5 to 4.6.

Ciprofloxacin ethylenediamine analog. Not more than 0.5 per cent, determined by the method described under Assay. Calculate the percentage of ciprofloxacin ethylenediamine analog from the chromatogram obtained with the test solution from the following expression.

$$\text{Per cent of the analog} = \frac{0.7 \times r_a}{0.7 \times (r_a + r_c)} \times 100$$

where 0.7 is the response factor for ciprofloxacin ethylenediamine analog relative to that of ciprofloxacin, r_a and r_c are the responses of ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak respectively.

Lactic acid. 0.288 mg to 0.352 mg for each mg of Ciprofloxacin stated on the label.

Determine by liquid chromatography (2.4.14).

Test solution. The substance under examination.

Reference solution. A 0.08 per cent w/v solution of *sodium lactate IPRS* in *water*.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 to 11 µm),
- column temperature: 40° ± 1°,
- mobile phase: a mixture of 85 volumes of *0.0025 M sulphuric acid* and 15 volumes of *acetonitrile*,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 208 nm,
- injection volume: 20 µl.

Inject the reference solution and record the chromatograms adjusting the sensitivity and flow rate suitably so that the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution, record the chromatograms and measure the peak responses for the major peaks. Calculate the content of lactic acid, $C_3H_6O_3$, in the substance under examination.

NOTE — After each analysis, the column should be rinsed with a mixture of 85 volumes of 0.005 M sulphuric acid and 15 volumes of acetonitrile to elute the ciprofloxacin from the column. The column may be regenerated with 0.005 M sulphuric acid and may be reused or stored.

Dextrose *(if present)*. 4.75 per cent to 5.25 per cent w/v of $C_6H_{12}O_6,H_2O$, determined by the following method. To 50.0 ml add 0.2 ml of *6 M ammonia* and dilute to 100.0 ml. Mix well and determine the optical rotation at 25° in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 2.085 represents the percentage of dextrose monohydrate, $C_6H_{12}O_6,H_2O$, in the preparation under examination.

Sodium chloride *(if present)*. 0.855 per cent to 0.945 per cent w/v of NaCl, determined by the following method. To 10.0 ml add 150 ml of *water* and titrate with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per mg of ciprofloxacin.

Sterility (2.2.11). Complies with the test for sterility, using Method A.

Particulate contamination (2.5.9). Complies with the limit test for particulate contamination.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing 25 mg of Ciprofloxacin to 100.0 ml with the mobile phase and mix.

Reference solution (a). A 0.03 per cent w/v solution of *ciprofloxacin hydrochloride IPRS* in the mobile phase.

Reference solution (b). Dissolve a sufficient quantity of *ciprofloxacin ethylenediamine analog IPRS* in reference solution (a) so as to obtain a solution containing 0.025 per cent w/v of the reference substance.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperaure: 30° ± 1°,
- mobile phase: a mixture of 87 volumes of *0.025 M phosphoric acid*, previously adjusted to pH 3.0 ± 0.1 with *triethyl-amine* and 13 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume: 10 µl.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the

resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6.

Inject reference solution (a). The column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

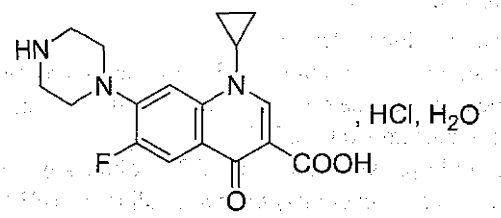
Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the injection.

Storage. Store protected from light at a temperature not exceeding 30°. The contents should not be allowed to freeze.

Labelling. The label states whether Dextrose or Sodium Chloride has been used for preparing the injection.

Ciprofloxacin Hydrochloride



$C_{17}H_{18}FN_3O_3,HCl,H_2O$ Mol. Wt. 385.8

Ciprofloxacin Hydrochloride is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid hydrochloride monohydrate.

Ciprofloxacin Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{18}FN_3O_3,HCl$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ciprofloxacin hydrochloride IPRS* or with the reference spectrum of ciprofloxacin hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber.

Mobile phase. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia* solution and 10 volumes of *acetonitrile*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *water*.

Reference solution. A 1 per cent w/v solution of *ciprofloxacin hydrochloride IPRS* in *water*.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine under ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. It gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 3.0 to 4.5, determined in a 2.5 per cent w/v solution.

Related substances. Carry out the method described under Assay and calculate the percentage of each impurity peak in the chromatogram obtained with the test solution. The content of ciprofloxacin ethylenediamine analog or of any other individual impurity peak found is not more than 0.2 per cent and the sum of all the impurity peaks is not more than 0.5 per cent.

Fluoroquinolonic acid. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia* solution and 10 volumes of *acetonitrile*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *water*.

Reference solution. Weigh 10 mg of *fluoroquinolonic acid IPRS*, add 0.1 ml of *6 M ammonia* and dilute to 100.0 ml with *water*. Dilute 2.0 ml of the solution to 10.0 ml with *water*.

Apply to the plate 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes. Remove the plate and place it in a chamber containing the mobile phase. After development, dry the plate in air for 15 minutes and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the spot of fluoroquinolonic acid is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphates (2.3.17). 0.375 g complies with the limit test for sulphates (400 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 4.7 to 6.7 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 50 mg of the substance under examination and dissolve in 100.0 ml of *water.*

Reference solution (a). A 0.05 per cent w/v solution of *ciprofloxacin hydrochloride IPRS* in *water.*

Reference solution (b). A 0.05 per cent w/v solution of *ciprofloxacin ethylenediamine analog IPRS* in *reference solution (a).*

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 87 volumes of *0.025 M phosphoric acid*, previously adjusted to pH 3.0 ± 0.1 with *triethylamine* and 13 volumes of *acetonitrile,*
- flow rate: 1.5 ml per minute,
- column temperature: 30° ± 1°,
- spectrophotometer set at 278 nm,
- injection volume: 10 µl.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6.

Inject reference solution (a). The test is not valid unless the column efficiency, determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{18}FN_3O_3$,HCl.

Storage. Store protected from light.

Ciprofloxacin Eye Drops

Ciprofloxacin Hydrochloride Eye Drops

Ciprofloxacin Eye Drops are a sterile solution of Ciprofloxacin Hydrochloride in Purified water.

Ciprofloxacin Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ciprofloxacin, $C_{17}H_{18}FN_3O_3$.

Usual strength. 0.3 per cent w/v.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

B. It give reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 5.5.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately measured volume of Eye drops containing 6 mg of ciprofloxacin, to a 50-ml volumetric flask, dilute with *water* to volume, and mix.

Reference solution (a). A 0.014 per cent w/v solution of *ciprofloxacin hydrochloride IPRS* in *water.*

Reference solution (b). A 0.001 per cent w/v solution of *ciprofloxacin ethylenediamine analog IPRS* in *reference solution (a).*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: a mixture of 75 volumes of *0.005 M tetrabutylammonium phosphate*, adjusted to pH 2.0 with *orthophosphoric acid* and 25 volumes of *methanol,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject reference solution (b). The relative retention time are about 0.8 for the ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak is not less than 1.5.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 500 theoretical plates, the tailing factor not more than 2.0, and the relative standard deviation for replicate injections is not more than 2 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the eye drops.

Storage. Store protected from light.

Ciprofloxacin Tablets

Ciprofloxacin Hydrochloride Tablets

Ciprofloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ciprofloxacin, $C_{17}H_{18}FN_3O_3$.

Usual strengths. The equivalent of 250 mg; 500 mg; 750 mg of ciprofloxacin.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber.

Mobile phase. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *acetonitrile*.

Test solution. Shake a quantity of the powdered tablets containing about 0.15 g of ciprofloxacin with 75 ml of *water* for 20 minutes, dilute to 100.0 ml with *water*, mix, centrifuge and use the clear supernatant liquid.

Reference solution. A 0.15 per cent w/v solution of *ciprofloxacin hydrochloride IPRS* in *water*.

Apply to the plate, as 1-cm bands, 5 µl of each solution. Place the plate in an atmosphere of ammonia for about 15 minutes and transfer it to an unsaturated chamber containing the mobile phase. Allow the mobile phase to rise 12 cm. Dry the plate in air for 15 minutes and examine under ultraviolet light at 254 nm and at 365 nm. The principal band in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with *water* if necessary, at the maximum at about 276 nm (2.4.7). Calculate the content of ciprofloxacin, $C_{17}H_{18}FN_3O_3$, in the medium from the absorbance obtained by repeating the determination using a solution of known concentration of *ciprofloxacin hydrochloride IPRS*.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{18}FN_3O_3$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 1.25 g of ciprofloxacin, add about 400 ml of *0.01 M hydrochloric acid*, shake for 20 minutes, dilute to 500.0 ml with *0.01 M hydrochloric acid*. and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *0.01 M hydrochloric acid*.

Reference solution (a). A 0.03 per cent w/v solution of *ciprofloxacin hydrochloride IPRS* in *0.01 M hydrochloric acid*.

Reference solution (b). A 0.05 per cent w/v solution of *ciprofloxacin ethylenediamine analog IPRS* in *reference solution (a)*.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30° ± 1°,
- mobile phase: a mixture of 87 volumes of *0.025 M phosphoric acid*, previously adjusted to pH 3.0 ± 0.1 with *triethylamine* and 13 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume: 10 µl.

Inject reference solution (b) and record the chromatogram adjusting the sensitivity and flow rate suitably so that the retention time for ciprofloxacin is between 6.4 and 10.8 minutes, the relative retention times are about 0.7 for ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin and the resolution between ciprofloxacin ethylenediamine analog peak and ciprofloxacin peak is not less than 6.

Inject reference solution (a). The column efficiency determined from ciprofloxacin peak, is not less than 2500 theoretical plates, the tailing factor for the ciprofloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

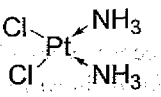
Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{18}FN_3O_3$ in the tablets.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of ciprofloxacin.

Cisplatin



$H_6Cl_2N_2Pt$ Mol. Wt. 300.0

Cisplatin is *cis*-diamminedichloroplatinum(II).

Cisplatin contains not less than 97.0 per cent and not more than 102.0 per cent of $H_6Cl_2N_2Pt$.

Description. A yellow powder or orange yellow crystals.

CAUTION — Cisplatin is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.

NOTE — Carry out all the tests and the Assay, except Identification tests A and C and the test for Silver, protected from light.

Category. Anticancer.

Identification

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cisplatin IPRS* or with the reference spectrum of cisplatin.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *cellulose*.

Mobile phase. A mixture of 10 volumes of *acetone* and 90 volumes of *dimethylformamide*.

Test solution. A 0.2 per cent w/v solution of the substance under examination in *dimethylformamide*.

Reference solution. A 0.2 per cent w/v solution of *cisplatin IPRS* in *dimethylformamide*.

Activate the plate by heating at 150° for 1 hour. Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and spray with a 5.0 per cent w/v solution of *stannous chloride* in a mixture of equal volumes of *dilute hydrochloric acid* and *water*. Examine after 1 hour. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Add 50 mg to 2 ml of *2 M sodium hydroxide*, evaporate to dryness, dissolve the residue in a mixture of 0.5 ml of *nitric acid* and 1.5 ml of *hydrochloric acid* and evaporate to dryness again; the residue is orange. Dissolve the residue in 0.5 ml of *water* and add 0.5 ml of *ammonium chloride solution*; a yellow crystalline precipitate is produced.

Tests

Solution A. A 0.1 per cent w/v solution in 0.9 per cent w/v solution of *sodium chloride* in *carbon dioxide-free water*.

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution GYS5 (2.4.1). A 2.0 per cent w/v solution in *dimethylformamide* is clear (2.4.1).

pH (2.4.24). 4.5 to 6.0, determined in solution A, measured immediately after preparation.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light. Do not heat or sonicate any platinum-containing solution. All solutions are to be used within 4 hours.

Saline solution. A 0.9 per cent w/v solution of *sodium chloride* in *water*.

Test solution. Dissolve 25 mg of the substance under examination in saline solution and dilute to 25.0 ml with saline solution.

Reference solution (a). A 0.1 per cent w/v solution of *cisplatin IPRS* in saline solution.

Reference solution (b). A solution containing 0.0002 per cent w/v of the substance under examination, 0.002 per cent w/v of *cisplatin impurity A IPRS* and 0.00112 per cent w/v of *cisplatin impurity B IPRS* in saline solution.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 20.0 ml with saline solution.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with base deactivated octylsilane bonded to porous silica (4 µm),
- mobile phase: dissolve 1.08 g of *sodium octanesulphonate*, 1.7 g of *tetrabutylammonium hydrogen sulphate* and 2.72 g of *potassium dihydrogen phosphate* in *water* and dilute to 950 ml with *water*, adjusted to pH 5.9 with *1 M sodium hydroxide* and dilute to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Name	Relative retention time
Displacement peak	0.5
Cisplatin impurity A[1]	0.6
Cisplatin impurity B[2]	0.7
Cisplatin (retention time: about 3.8 minutes)	1.0
Cisplatin aquo complex	1.2

[1]transplatin,

[2]amminetrichloroplatinate.

The displacement peak is the latest eluting peak of the group of injection peaks in the chromatogram obtained with the blank solution.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cisplatin impurities A and B is not less than 2.5. The displacement peak and the peak due to cisplatin impurity A are well separated.

Inject reference solution (b) and (c) and the test solution. Run the chromatogram 7 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to cisplatin impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (2.0 per cent). The area of any peak corresponding to cisplatin impurity B is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of the peak due to cisplatin in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks other than cisplatin impurities A and B is not more than 2.5 times the area of the peak due to cisplatin in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than the area of the peak in the chromatogram obtained with reference solution (c) (0.05 per cent) and due to the cisplatin aquo complex.

Silver. Not more than 250 ppm.

Determine by atomic absorption spectrophotometry (2.4.2), measuring at 328 nm using air-acetylene flame and silver hollow-cathode lamp using a transmission band of 0.5 nm.

Test solution. Dissolve 0.1 g in 15 ml of *nitric acid*, heating at 80°. Cool and dilute to 25.0 ml with *water*.

Reference solutions. To suitable volumes (10 ml to 30 ml) of *silver standard solution (5 ppm Ag)*, add 50 ml of *nitric acid* and dilute to 100.0 ml with *water*.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances with the following modifications.

- injection volume: 10 µl.

Inject reference solution (a) and the test solution.

Calculate the content of $H_6Cl_2N_2Pt$.

Storage. Store protected from light and moisture.

Cisplatin Injection

Cisplatin Injection is a sterile solution of Cisplatin in Water for Injections. It is either supplied as a ready-to-use solution or it is prepared by dissolving Cisplatin for Injection in the requisite amount of Water for Injections immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

When supplied as a ready-to-use solution, the injection complies with the following requirements.

Cisplatin Injection contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of cisplatin, $Cl_2H_6N_2Pt$.

Usual strengths. 50 mg per 50 ml; 10 mg per 20 ml.

Description. A clear, colourless to pale yellow solution.

NOTE — Except identification test A, carry out the tests protected from light.

Identification

A. When examined in the range 230 nm to 350 nm (2.4.7) of a solution diluted, if necessary to contain a 0.1 per cent w/v of Cisplatin shows an absorption maximum at 300 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 3.5 to 6.5.

Trichloroammineplatinate. Determine by liquid chromatography (2.4.14).

NOTE — prepare the solutions immediately before use and protect from light.

Saline solution. A 0.9 per cent w/v *sodium chloride* in *water*.

Test solution. Dilute the injection with saline solution to obtain a solution containing 0.05 per cent w/v of Cisplatin.

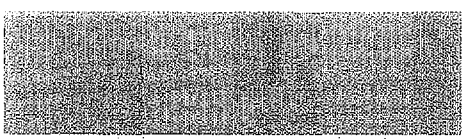
Reference solution. Dissolve a quantity of *potassium trichloroammineplatinate IPRS* in saline solution to obtain a solution containing 0.0015 per cent w/v of trichloroammineplatinate.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica chemically bonded with strongly basic quaternary ammonium anion-exchange coating (10 µm) (Such as Spherisorb SAX),
- mobile phase: a 0.04 per cent w/v solution of *ammonium sulphate*, adjusted to pH between pH 5.8 to 6.0,
- flow rate: 2 ml per minute,
- spectrophotometer set at 209 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between sodium chloride and trichloroammineplatinate is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to trichloroammineplatinate is not more than the area of the principal peak in the chromatogram obtained with the reference solution (3.0 per cent).



Transplatin. Determine by liquid chromatography (2.4.14).

Saline solution. A 0.9 per cent w/v *sodium chloride* in *water*.

Test solution. Prepare in the same manner as reference solution (a) but using 10 ml of the injection, diluted if necessary, with saline solution to produce a solution containing 0.05 per cent w/v of Cisplatin in place of the 10 ml of solution A.

Reference solution (a). Add 10 ml of a 0.005 per cent w/v solution of *transplatin IPRS* in saline solution to 25 mg of *cisplatin IPRS*, dilute to 25 ml with saline solution, shake for 30 minutes to effect dissolution and add sufficient saline solution to produce 50 ml (Solution A). Mix 5 ml of a freshly prepared 0.5 per cent w/v solution of *thiourea*, 5 ml of *1 M hydrochloric acid* and 10 ml of solution A, heat an aliquot in a reaction vial at 60° for 1 hour and cool.

Reference solution (b). Prepare in the same manner as reference solution (a) but using a mixture of 10 ml of a solution containing 0.005 per cent w/v of *cisplatin IPRS* in saline solution and 10 ml of a 0.005 per cent w/v solution of *transplatin IPRS* in saline solution in place of 10 ml of solution A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with silica chemically bonded with strongly acidic cation-exchange coating (10 µm) (Such as Maxsil SCX),
- column temperature: 45°,
- mobile phase: a 2.5 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 3.2 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- Injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cisplatin and transplatin is not less than 1.7 and the column efficiency due to transplatin peak is not less than 2500 theoretical plates.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of peak due to transplatin is not more than the area of the principal peak in the chromatogram obtained with the reference solution (a) (2.0 per cent).

Bacterial endotoxins (2.2.3). Not more than 2.0 Endotoxin Units per mg of Cisplatin.

Assay. Determine by liquid chromatography (2.4.14).

Saline solution. A 0.9 per cent w/v *sodium chloride* in *water*.

Test solution. Dilute the injection with saline solution to obtain a solution of 0.1 per cent w/v of Cisplatin.

Reference solution (a). A 0.1 per cent w/v solution of *cisplatin IPRS* in saline solution.

Reference solution (b). A solution containing 0.05 per cent w/v of *cisplatin IPRS* and 0.005 per cent w/v of *transplatin IPRS* in saline solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with silica chemically bonded with amine groups (10 µm) (Such as Lichrosorb NH_2),
- mobile phase: a mixture of 10 volumes of *water* and 90 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cisplatin and transplatin is not less than 3.5.

Inject reference solution (a) and the test solution.

Calculate the content of $Cl_2H_6N_2Pt$ in injection.

Storage. Store protected from light. It should not be refrigerated.

Cisplatin for Injection

Cisplatin for Injection is a freeze dried mixture of Cisplatin, Mannitol and Sodium Chloride. It is supplied in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Cisplatin for Injection contains Cisplatin not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cisplatin, $Cl_2H_6N_2Pt$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for injection) and with the following requirements.

NOTE — With the exception of Identification test A, carry out the tests protected from light.

Identification

A. When examined in the range 230 nm to 350 nm (2.4.7) of a solution containing a 0.1 per cent w/v of Cisplatin in *0.1M hydrochloric acid* shows an absorption maximum at 300 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 3.5 to 6.5 in a solution containing 0.1 per cent w/v of Cisplatin.

NOTE — Prepare the solutions immediately before use and protect from light.

Trichloroammineplatinate. Complies with the test described for ready-to-use solution with the following modification.

Test solution. Dissolve the contents of a sealed container in saline solution to obtain a solution containing 0.05 per cent w/v of Cisplatin.

Transplatin. Complies with the test described for ready-to-use solution with the following modification.

Test solution. Prepare in the same manner as reference solution (a) but using 10 ml of a solution prepared by shaking the contents of a sealed container with sufficient saline solution for 30 minutes to produce a solution containing 0.1 per cent w/v of Cisplatin in place of 10 ml of solution A.

Bacterial endotoxins (2.2.3). Not more than 2.0 Endotoxin Units per mg of Cisplatin.

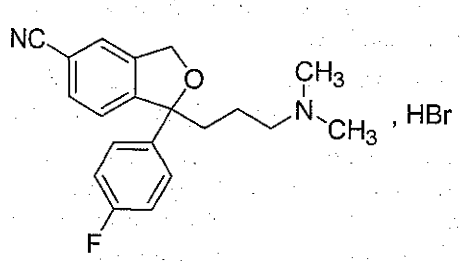
Assay. Complies with the test described for ready-to-use solution with the following modification.

Test solution. Dissolve the contents of a sealed container in saline solution to produce a solution containing 0.1 per cent w/v of Cisplatin.

Calculate the content of $Cl_2H_6N_2Pt$ in the injection.

Storage. Store protected from light. It should not be refrigerated.

Citalopram Hydrobromide



, HBr

$C_{20}H_{21}FN_2O,HBr$ Mol.Wt. 405.3

Citalopram Hydrobromide is (*RS*)-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide.

Citalopram Hydrobromide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{21}FN_2O,HBr$, calculated on the anhydrous basis.

Category. Antidepressent.

Description. A white to off-white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *citalopram hydrobromide IPRS* or with the reference spectrum of citalopram hydrobromide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives the reactions of bromides (2.3.1).

Tests

pH (2.4.24). 5.5 to 6.5, determined in a 0.5 per cent w/v solution in *water*.

Optical rotation (2.4.22). – 0.1° to + 0.1°, determined at 20°, in a 5 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *methanol* and 50 volumes of *water*.

Test solution. Dissolve 62.5 mg of the substance under examination in 100.0 ml of the solvent mixture and filter.

Reference solution (a). A 0.625 µg per ml solution of *citalopram hydrobromide IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0001 per cent w/v, each of, *citalopram hydrobromide IPRS* and *citalopram impurity A IPRS [[1-(4- Fluorophenyl)-1-(-3-(methylamino [propyl-1,dihydroiso-benzofuran-5-carbonitrile hydrochloride] IPRS]* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 1.0 g of *sodium acetate* in 800 ml of *water*, adding 6 ml of *triethylamine*, adjusted to pH 4.6 with *acetic acid*, and diluting to 1000 ml with *water*, and 20 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume: 20 µl.

The relative retention time for impurity A with respect to citalopram is about 0.9.

Inject reference solution (b). The test is not valid unless the resolution between impurity A and citalopram is not less than 1.8, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.25 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *methanol* and 50 volumes of *water*.

Test solution. Weigh accurately about 62.5 mg of the substance under examination, dissolve in 100.0 ml of the solvent mixture and filter.

Reference solution. A 0.0625 per cent w/v solution of *citalopram hydrobromide IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 1.0 g of *sodium acetate* in 800 ml of *water*, adding 6 ml of *triethylamine*, adjusted to pH 4.6 with *acetic acid and* diluting to 1000 ml with *water*, and 20 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}FN_2O$, HBr.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Citalopram Tablets

Citalopram Hydrobromide Tablets

Citalopram Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of citalopram, $C_{20}H_{21}FN_2O$.

Usual strengths. 10 mg; 20 mg; 40 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of a buffer solution prepared by mixing 118 ml of *1 M hydrochloric acid* and 82 ml of *1 M sodium hydroxide* and diluting to 1000 ml with *water*. Adjusted to pH 1.5 with *1 M sodium hydroxide*,

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 239 nm (2.4.7). Calculate the content of $C_{20}H_{21}FN_2O$ in the medium from the absorbance obtained from a solution of known concentration of *citalopram hydrobromide IPRS* in the same medium.

Q. Not less than 80 per cent of the stated amount of $C_{20}H_{21}FN_2O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the powdered tablets containing 50.0 mg of citalopram, disperse in 100.0 ml of the mobile phase and filter.

Reference solution (a). A 0.625 µg per ml solution of *citalopram hydrobromide IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0001 per cent w/v of *citalopram impurity B IPRS [[3-(3N,N-dimethylamino)-1-(4-fluorophenyl)-6-c vano-1(3H)-isobenzofuranone]IPRS]* and 0.025 per cent w/v of *citalopram hydrobromide IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 3.15 g of *potassium dihydrogen phosphate* and 3.6 g of *disodium hydrogen phosphate*

in 1000 ml of *water*, 38 volumes of *methanol* and 7 volumes of *acetonitrile*, adjusted to pH 6.5 with *orthophosphoric acid*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between impurity B and citalopram is not less than 3.0.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.25 per cent) and the sum of the areas of all the secondary peaks is not more than 8 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.8 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Use the chromatographic system described under Assay using the following test solution.

Test solution. Powder one tablet, disperse in 10 ml of a 0.142 per cent w/v solution of *anhydrous dibasic sodium phosphate*, add 40 ml of *methanol* and mix with the aid of ultrasound for 5 minutes. Add sufficient volume of the internal standard solution and dilute stepwise, if necessary with the solvent mixture to obtain a solution containing 0.01 per cent w/v of citalopram and 0.0025 per cent w/v of internal standard solution and filter.

Calculate the content of $C_{20}H_{21}FN_2O$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *methanol* and 20 volumes of a 0.142 per cent w/v solution of *anhydrous dibasic sodium phosphate*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 100 mg of citalopram, disperse in 100.0 ml of the solvent mixture and filter. To 5.0 ml of the solution, add 5.0 ml of the internal standard solution and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.125 per cent w/v solution of *citalopram hydrobromide IPRS* (equivalent to 0.1 per cent w/v of citalopram) in the solvent mixture. To 5.0 ml of the solution, add 5.0 ml of the internal standard solution and dilute to 50.0 ml with the solvent mixture.

Internal standard solution. A 0.025 per cent w/v solution of *dimethyl-(1-methyl-3,3-diphenylallyl)amine hydrochloride IPRS* (*citalopram impurity C IPRS*) in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a 0.077 per cent w/v solution of *dodecyltrimethylammonium bromide* in the solvent mixture,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The relative retention time for citalopram impurity C is about 1.36 and the resolution between citalopram and citalopram impurity C is not less than 1.5. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 1.5 per cent.

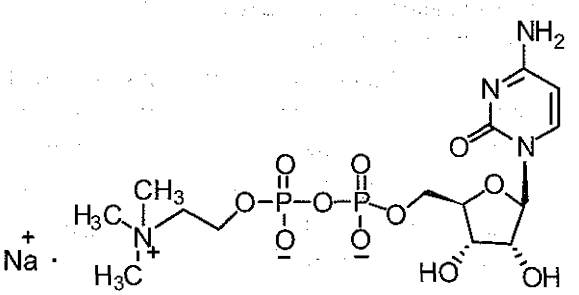
Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}FN_2O$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of citalopram.

Citicoline Sodium



$C_{14}H_{25}N_4NaO_{11}P_2$ Mol. Wt. 510.3

Citicoline Sodium is Cytidine-5'-diphosphocholine sodium.

Citicoline Sodium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{25}N_4NaO_{11}P_2$, calculated on the anhydrous basis.

Category. Nootropic.

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *citicoline sodium IPRS* or with the reference spectrum of citicoline sodium.

B. It gives the reactions of sodium salt (2.3.1).

Tests

pH (2.4.24). 6.5 to 7.5, determined on 20 per cent w/v solution in *water*.

Related substances. Determined by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in *water* and dilute to 50.0 ml with *water*.

Reference solution (a). A 0.001 per cent w/v solution of *citicoline sodium IPRS* in *water*.

Reference solution (b). A solution containing 0.2 per cent w/v of *citicoline sodium IPRS* and 0.002 per cent w/v of *citicoline impurity B IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- sample temperature: 10°,
- mobile phase: A. a mixture of 1 volume of *methanol* and 99 volumes of 0.2 per cent v/v solution of *formic acid*, adjusted to pH 7.5 with *triethylamine*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume: 10 µl.

Time (in mins.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
20	100	0
40	75	25
45	75	25
47	100	0
60	100	0

Name	Relative retention time	Correction factor
Citicoline impurity A[1]	1.2	—
Citicoline impurity B[2]	1.57	0.67
Citicoline impurity C[3]	3.62	0.64
Citicoline impurity D[4]	4.00	1.00

[1]desmethyl cytidine-5'-diphosphocholine sodium,

[2]cytidine-5'-monophosphate; 5-CMP,

[3]methyl ester of cytidine-5'-monophosphate; 5-CMP ester,

[4]cytidine-5'-monophosphomorpholidate; 5-CMP morpholidate.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 6000 theoretical plates and the tailing factor is not more than 1.5.

Inject reference solution (b). The test is not valid unless the resolution between the citicoline impurity B and principal peak is not less than 7.0.

Inject reference solution (a) and the test solution. The area of any secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 2 times the area of principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Iron (2.3.14). 4 g complies with the limit test for iron (10 ppm).

Chlorides (2.3.12). 0.5 g complies with the limit test for chlorides (500 ppm).

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Heavy metals (2.3.13). 2 g complies with the limit test for heavy metals, Method B (10 ppm).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *water* and dilute to 100.0 ml with *water*.

Reference solution. A 0.05 per cent w/v solution of *citicoline sodium IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- sample temperature: 10°,
- mobile phase: a mixture of 1 volume of *methanol* and 99 volumes of 0.2 per cent v/v solution of *formic acid*, adjusted to pH 7.5 with *triethylamine*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{25}N_4NaO_{11}P_2$.

Storage. Store protected from moisture.

Citicoline Injection

Citicoline Sodium Injection

Citicoline Injection is a sterile solution of Citicoline Sodium in Water for Injections.

Citicoline Injection contains Citicoline Sodium equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of citicoline, $C_{14}H_{26}N_4O_{11}P_2$.

Usual strength. 250 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.3 to 8.0.

Related Substances. Determine by liquid chromatography (2.4.14).

NOTE—Determine water content of citicoline sodium IPRS before use and calculate the potency.

Test solution. Dilute a volume of injection containing 0.25 g of citicoline to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase and filter.

Reference solution (a). Dissolve a quantity of *citicoline sodium IPRS* in the mobile phase to obtain a solution containing 0.0005 per cent w/v of citicoline.

Reference solution (b). A solution containing 0.00025 per cent, w/v each, of *citicoline sodium IPRS* and *5'-cytidylic acid IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 95 volumes of buffer solution prepared by dissolving 1.697 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, add 2 ml of *triethylamine* and adjusted to pH 6.0 with *dilute acetic acid* and 5 volumes of *methanol*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Equilibrate the column with mobile phase for at least 90 minutes.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the principal peak and 5'-cytidylic acid peak is not less than 6.0 in the chromatogram obtained with reference solution (b) and the tailing factor is not more than 2.0, the relative standard deviation for replicate injections is not more than 3.0 per cent in the chromatogram obtained with the reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of peak due to 5'-cytidylic acid multiplying by correction factor of 0.7 is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks other than the 5'-cytidylic acid peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 0.175 Endotoxin Unit per mg of Citicoline.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Determine water content of citicoline sodium IPRS before use and calculate the potency

Test solution. Mix the content of 10 containers. Dilute a volume of the injection containing 0.25 g of citicoline with the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase and filter.

Reference solution. Dissolve a quantity of *citicoline sodium IPRS* in the mobile phase to obtain a solution containing 0.005 per cent w/v of citicoline.

Use chromatographic system as described under Related substances.

Equilibrate the column with mobile phase for at least 90 minutes.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{26}N_4O_{11}P_2$ in the injection.

Storage. Store protected from light, at a temperature not exceeding 25°.

Labelling. The label states (1) the strength in terms of the equivalent amount of citicoline; (2) the preparation is intended for intramuscular and intravenous injection only.

Citicoline Prolonged-release Tablets

Citicoline Sustained-release Tablets; Citicoline Extended-release Tablets; Citicoline Sodium Prolonged-release Tablets; Citicoline Sodium Sustained-release Tablets; Citicoline Sodium Extended-release Tablets

Citicoline Prolonged-release Tablets contain Citicoline Sodium.

Citicoline Prolonged-release Tablets are manufactured by different manufacturers, whilst complying with the

requirements of the monograph are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Citicoline Prolonged-release Tablets contain Citicoline Sodium equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of citicoline, $C_{14}H_{26}N_4O_{11}P_2$.

Usual strength. 1000 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Determine water content of citicoline sodium IPRS before use and calculate the potency.

Test solution. Disperse a quantity of the powdered tablets containing 100 mg of citicoline in the mobile phase with the aid of ultrasound for 30 minutes and dilute to 200.0 ml with the mobile phase.

Reference solution (a). Dissolve a quantity of *citicoline sodium IPRS* in the mobile phase to obtain a solution containing 0.0005 per cent w/v of citicoline.

Reference solution (b). A solution containing 0.00025 per cent w/v, each of, *citicoline sodium IPRS* and *5'-cytidylic acid IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5µm),
- column temperature: 30°,
- mobile phase: a mixture of 95 volumes of buffer solution prepared by dissolving 1.697 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, add 2 ml of *triethylamine* and adjusted to pH 6.0 with *dilute acetic acid* and 5 volumes of *methanol*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Equilibrate the column with mobile phase for at least 90 minutes.

Inject reference solution (a) and (b). The test is not valid unless the tailing factor is not more than 2.0, the relative standard deviation for replicate injections is not more than 3.0 per cent in the chromatogram obtained with the reference solution (a) and the resolution between the principal peak and 5'-cytidylic acid peak is not less than 6.0 in the chromatogram obtained with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of peak due to 5'-cytidylic acid multiplying by correction factor of 0.7 is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks other than the 5'-cytidylic acid peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Determine water content of citicoline sodium IPRS before use and calculate the potency.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 100 mg of citicoline in the mobile phase with the aid of ultrasound for 30 minutes and dilute to 200.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve a quantity of *citicoline sodium IPRS* in the mobile phase and dilute to obtain a solution containing 0.005 per cent w/v of citicoline.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: a mixture of 95 volumes of buffer solution prepared by dissolving 1.697 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, add 2 ml of *triethylamine* and adjusted to pH 6.0 with *dilute acetic acid* and 5 volumes of *methanol*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for at least 90 minutes.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{26}N_4O_{11}P_2$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 25°.

Labelling. The label states the strength in terms of the equivalent of amount of citicoline.

Citicoline Tablets

Citicoline Sodium Tablets

Citicoline Tablets contain Citicoline Sodium.

Citicoline Tablets contain Citicoline Sodium equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of citicoline, $C_{14}H_{26}N_4O_{11}P_2$.

Usual strengths. 100 mg; 250 mg; 500 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate if necessary, with dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_{14}H_{25}N_4O_{11}P_2$ in the medium from the absorbance obtained from a solution of known concentration of *citicoline sodium IPRS.*

Q. Not less than 75 per cent of the stated amount of $C_{14}H_{26}N_4O_{11}P_2$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Determine water content of citicoline sodium IPRS before use and calculate the potency.

Test solution. Disperse a quantity of the powdered tablets containing 100 mg of citicoline in the mobile phase with the aid of ultrasound for 30 minutes and dilute to 200.0 ml with the mobile phase and filter.

Reference solution (a). Dissolve a quantity of *citicoline sodium IPRS* in the mobile phase to obtain a solution containing 0.0005 per cent w/v of citicoline.

Reference solution (b). A solution containing 0.00025 per cent w/v, each of, *citicoline sodium IPRS* and *5'-cytidylic acid IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 95 volumes of buffer solution prepared by dissolving 1.697 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, add 2 ml of *triethylamine* and adjusted to pH 6.0 with *dilute acetic acid* and 5 volumes of *methanol*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Equilibrate the column with mobile phase for at least 90 minutes.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the principal peak and 5'-cytidylic acid peak is not less than 6.0 in the chromatogram obtained with reference solution (b) and the tailing factor is not more than 2.0, the relative standard deviation for replicate injections is not more than 3.0 per cent in the chromatogram obtained with the reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of peak due to 5'-cytidylic acid multiplying by correction factor of 0.7 is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks other than the 5'-cytidylic acid peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Determine water content of citicoline sodium IPRS before use and calculate the potency.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 100 mg of citicoline in the mobile phase with the aid of ultrasound for 30 minutes and dilute to 200.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase and filter.

Reference solution. Dissolve a quantity of *citicoline sodium IPRS* in the mobile phase to obtain a solution containing 0.005 per cent w/v of citicoline.

Use chromatographic system as described under Related substances.

Equilibrate the column with mobile phase for at least 90 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

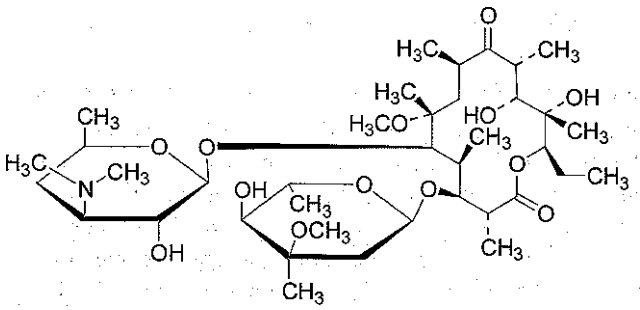
Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{26}N_4O_{11}P_2$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 25°.

Labelling. The label states the strength in terms of the equivalent of amount of citicoline.

Clarithromycin



$C_{38}H_{69}NO_{13}$ Mol. Wt. 748.0

Clarithromycin is (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-α-D-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (6-O-methylerythromycin A).

Clarithromycin contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{38}H_{69}NO_{13}$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clarithromycin IPRS* or with the reference spectrum of clarithromycin.

Tests

Specific optical rotation (2.4.22). –102° to – 94°, determined on a 1 per cent w/v solution in *dichloromethane* at 20°.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 75 mg of the substance under examination in 25 ml of *acetonitrile* and dilute to 50.0 ml with *water*.

Reference solution (a). Dissolve 7.5 mg of *clarithromycin IPRS* in 2.5 ml of *acetonitrile* and dilute to 5.0 ml with *water*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with equal volumes of *acetonitrile* and *water*.

Use chromatographic system as described under Assay.

Inject reference solution (b). the test is not valid unless the tailing factor is not more than 2.0.

Name	Relative retention time	Correction factor
Clarithromycin impurity I[1]	0.38	—
Clarithromycin impurity A[2]	0.42	—
Clarithromycin impurity J[3]	0.63	—
Clarithromycin impurity L[4]	0.74	—
Clarithromycin impurity B[5]	0.79	—
Clarithromycin impurity M[6]	0.81	—
Clarithromycin impurity C[7]	0.89	—
Clarithromycin impurity D[8]	0.96	—
Clarithromycin	1.0	—
Clarithromycin impurity N[9]	1.15	—
Clarithromycin impurity E[10]	1.27	—
Clarithromycin impurity F[11]	1.33	—
Clarithromycin impurity P[12]	1.35	—
Clarithromycin impurity O[13]	1.41	—
Clarithromycin impurity K[14]	1.59	—
Clarithromycin impurity G[15]	1.72	0.27
Clarithromycin impurity H[16]	1.82	0.15

[1]3-*O*-decladinosyl-6-*O*-methylerythromycin A,

[2]2-demethyl-2-(hydroxymethyl)-6-*O*- methylerythromycin A,

[3]erythromycin A (*E*)-9-oxime,

[4]6-*O*-methylerythromycin A (*Z*)-9-oxime,

[5]6-*O*-methyl-15-norerythromycin A,

[6]3"-*N*-demethyl-6-*O*-methylerythromycin A (*E*)-9-oxime,

[7]6-*O*-methylerythromycin A (*E*)-9-oxime,

[8]3"-*N*-demethyl-6-*O*-methylerythromycin A,

[9](10*E*)-10,11-didehydro-11-deoxy-6-*O*-methylerythromycin A,

[10]6,11-di-*O*-methylerythromycin A,

[11]6,12-di-*O*-methylerythromycin A,

[12]4',6-di-*O*-methylerythromycin A,

[13]6-*O*-methylerythromycin A (*Z*)-9-(*O*-methyloxime),

[14](1*S*,2*R*,5*R*,6*S*,7*S*,8*R*,9*R*,11*Z*)-2-ethyl-6-hydroxy-9-methoxy-1,5,7,9,11,13-hexamethyl-8-[[3,4,6-trideoxy-3-(dimethylamino)-α-D-xylo-hexopyranosyl]oxy]-3,15- dioxabicyclo[10.2.1]pentadeca-11,13-dien-4-one (3-*O*-decladinosyl-8,9:10,11-dianhydro-6-*O*-methylerythromycin A-9,12-hemiketal,

[15]6-*O*-methylerythromycin A (*E*)-9-(*O*-methyloxime),

[16]3"-*N*-demethyl-3'-*N*-formyl-6-*O*-methylerythromycin A.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3.5 times the area of the peak in the chromatogram obtained with reference solution (b) (3.5 per cent). Ignore any peak with an area less than 0.2 times of the principal peak obtained with reference solution (b) (0.2 per cent).

Heavy metals (2.3.13). Dissolve 2 g in a mixture of 15 volumes of *water* and 85 volumes of *dioxan* and dilute to 20 ml with the same solvent mixture. 12 ml of the solution complies with limit test for heavy metals, Method D (20 ppm), using 10 ml of *lead standard solution (2 ppm Pb)* in the same solvent mixture.

Water (2.3.43). Not more than 2.0 per cent w/w, determined on 0.5 g using *methanol* as solvent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 75 mg of the substance under examination in 25 ml of *acetonitrile* and dilute to 50.0 ml with *water*.

Reference solution. Dissolve 15 mg of the *clarithromycin IPRS* in 5 ml of *acetonitrile* and dilute to 10.0 ml with *water*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3.5µm),
- column temperature: 40°,
- mobile phase: A. 0.476 per cent w/v solution of *potassium dihydrogen phosphate* adjusted to pH 4.4 with *dilute orthophosphoric acid* or a 4.5 per cent solution of *potassium hydroxide*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 10 µl.

Time (in mins.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
32	40	60
34	40	60
36	75	25
42	75	25

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{69}NO_{13}$.

Storage. Store protected from moisture.

Clarithromycin Tablets

Clarithromycin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clarithromycin, $C_{38}H_{69}NO_{13}$.

Usual strengths. 250 mg; 500 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. Use 900 ml of a solution containing 1000 volumes of a 1.361 per cent w/v solution of *sodium acetate* and 350 volumes of 0.1M *acetic acid*, adjusted to pH 5.0 with *0.1M acetic acid*, at a temperature of 37° ± 0.5°, as the medium,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Weigh a suitable quantity of *clarithromycin IPRS*, dissolve in *methanol*, dilute with dissolution medium to obtain a solution having a known concentration of about 0.125 mg per ml.

Use chromatographic system as described under Assay.

Calculate the content of $C_{38}H_{69}NO_{13}$ in the tablet.

Q. Not less than 75 per cent of the stated amount of $C_{38}H_{69}NO_{13}$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Disperse a quantity of powdered tablets containing 75 mg of Clarithromycin in 50.0 ml of the solvent mixture, filter.

Reference solution (a). Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A 0.0015 per cent w/v solution of *3"-N-demethyl-6-O-methylerythromycin A IPRS (clarithromycin impurity D IPRS)* in the test solution.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (Such as Kingsorb C18),
- column temperature: 40°,
- mobile phase: A. a 0.476 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to

pH 4.4 with *orthophosphoric acid* or *potassium hydroxide solution*,

B. *acetonitrile*,

- a gradient programme using the conditions given below,
- flow rate: 1.1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
32	40	60
34	40	60
36	75	25
42	75	25

Name	Relative retention time	Correction factor
Clarithromycin impurity I[1]	0.38	—
Clarithromycin impurity A[2]	0.42	—
Clarithromycin impurity J[3]	0.63	—
Clarithromycin impurity L[4]	0.74	—
Clarithromycin impurity B[5]	0.79	—
Clarithromycin impurity M[6]	0.81	—
Clarithromycin impurity C[7]	0.89	—
Clarithromycin impurity D[8]	0.96	—
Clarithromycin	1.0	—
Clarithromycin impurity N[9]	1.15	—
Clarithromycin impurity E[10]	1.27	—
Clarithromycin impurity F[11]	1.33	—
Clarithromycin impurity P[12]	1.35	—
Clarithromycin impurity O[13]	1.41	—
Clarithromycin impurity K[14]	1.59	—
Clarithromycin impurity G[15]	1.72	0.27
Clarithromycin impurity H[16]	1.82	0.15

[1]3-*O*-decladinosyl-6-*O*-methylerythromycin A,

[2]2-demethyl-2-(hydroxymethyl)-6-*O*- methylerythromycin A,

[3]erythromycin A (*E*)-9-oxime,

[4]6-*O*-methylerythromycin A (*Z*)-9-oxime,

[5]6-*O*-methyl-15-norerythromycin A,

[6]3"-*N*-demethyl-6-*O*-methylerythromycin A (*E*)-9- oxime,

[7]6-*O*-methylerythromycin A (*E*)-9-oxime,

[8]3"-*N*-demethyl-6-*O*-methylerythromycin A,

[9](10*E*)-10,11-didehydro-11-deoxy-6-*O*-methylerythromycin A,

[10]6,11-di-*O*-methylerythromycin A,

[11]6,12-di-*O*-methylerythromycin A,

[12]4',6-di-*O*-methylerythromycin A,

[13]6-*O*-methylerythromycin A (*Z*)-9-(*O*-methyloxime),

[14](1*S*,2*R*,5*R*,6*S*,7*S*,8*R*,9*R*,11*Z*)-2-ethyl-6-hydroxy-9-methoxy-1,5,7,9,11,13-hexamethyl-8-[[3,4,6-trideoxy-3-(dimethylamino)-α-D-xylo-hexopyranosyl]oxy]-3,15-dioxabicyclo[10.2.1]pentadeca-11,13-dien-4-one (3-*O*-decladinosyl-8,9:10,11- dianhydro-6-*O*-methylerythromycin A-9,12-hemiketal,

[15]6-*O*-methylerythromycin A (*E*)-9-(*O*-methyloxime),

[16]3"-*N*-demethyl-3'-*N*-formyl-6-*O*-methylerythromycin A.

Inject reference solution (a) and (c). The test is not valid unless the tailing factor of the principal peak is not more than 1.75 in the chromatogram obtained with reference solution (a) and in the chromatogram obtained with reference solution (c), the peak to valley ratio is not less than 3.0, where H_p is the height above the baseline of the peak due to clarithromycin impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to clarithromycin.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.5 per cent). Ignore any peak less than 0.2 times of the principal peak obtained with reference solution (b) (0.1 per cent) and the peaks eluting before impurity I and after impurity H.

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 1 g by drying in an oven at 110°, under vacuum, for 3 hours.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.125 g of Clarithromycin in 200.0 ml with *methanol* and filter. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

Reference solution. A 0.0625 per cent w/v solution of *clarithromycin IPRS* in *methanol*. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 65 volumes of *methanol* and 35 volumes of *0.067 M monobasic potassium phosphate* adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency in not less than 750 theoretical plates, the

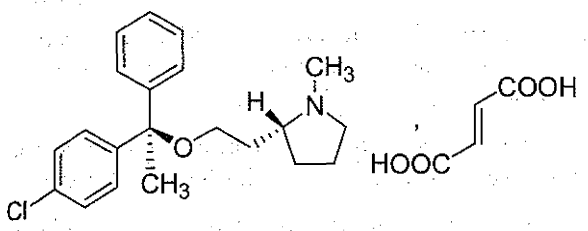
tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{69}NO_{13}$.

Storage. Store protected from moisture.

Clemastine Fumarate



$C_{21}H_{26}ClNO,C_4H_4O_4$ Mol. Wt.460.0

Clemastine Fumarate is (2*R*)-2-{2-[(1*R*)-1-(4-Chlorophenyl)-1-phenylethoxy]ethyl}-1-methylpyrrolidine fumarate.

Clemastine Fumarate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{21}H_{26}ClNO,C_4H_4O_4$, calculated on the dried basis.

Category. Antiallergic.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6), Compare the spectrum with that obtained with *clemastine fumarate IPRS* or with the reference spectrum of clemastine fumarate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 5 volumes of *water*, 25 volumes of *anhydrous formic acid* and 70 volumes of *di-isopropyl ether.*

Test solution. Dissolve 40 mg of the substance under examination in *methanol* and dilute to 2.0 ml of *methanol.*

Reference solution. A 0.5 per cent w/v solution of *fumaric acid IPRS* in *ethanol (95 per cent).*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air, heat at 105° for 30 minutes and spray with a 1.6 per cent w/v solution of *potassium permanganate* and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Solution A. A 1.0 per cent w/v solution in *methanol.*

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

pH (2.4.24). 3.2 to 4.2, determined in a 10.0 per cent w/v solution in *carbon dioxide-free water.*

Specific optical rotation (2.4.22). +15.0° to +18.0°, determined in solution A.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 1 volume of *13.5 M ammonia*, 20 volumes of *methanol* and 80 volumes of *tetrahydrofuran.*

Test solution (a) Dissolve 0.1 g of the substance under examination in *methanol* and dilute to 5.0 ml of *methanol.*

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol.*

Reference solution (a). A 0.2 per cent w/v solution of *clemastine fumarate IPRS* in *methanol.*

Reference solution (b). Dilute 1.5 ml of test solution (b) to 50.0 ml with *methanol.*

Reference solution (c). Dilute 0.5 ml of test solution (b) to 50.0 ml with *methanol.*

Reference solution (d). Dissolve 10 mg of *diphenhydramine hydrochloride IPRS* in 5.0 ml of reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in cold air for 5 minutes and spray with a freshly prepared mixture of 1.0 ml of *potassium iodobismuthate solution* and 10.0 ml of *dilute acetic acid* and then with *dilute hydrogen peroxide solution*, cover the plate immediately with a glass plate of the same size and examine the chromatograms after 2 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more than the principal spot in the chromatogram obtained with reference solution (b) (0.3 per cent), and at most 4 such spots are more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.1 per cent). Ignore any spot remaining at the point of application (*fumaric acid*). The spot in the chromatogram obtained with reference solution (d) shows 2 clearly separated spots.

Impurity C. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of 1.0 per cent w/v solution of *ammonium dihydrogen phosphate.*

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.006 per cent w/v solution of *clemastine impurity C IPRS (1-(4-chlorophenyl)-1-phenyl ethanol IPRS)* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture. To 1.0 ml of the solution, add 1.0 ml of reference solution (a) and dilute to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.1 volume of *orthophosphoric acid*, 45 volumes of *acetonitrile* and 55 volumes of 1.0 per cent w/v solution of *ammonium dihydrogenphosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 100 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to clemastine and clemastine impurity C is not less than 2.2.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to clemastine impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 6 hours.

Assay. Dissolve 0.35 g in 60 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*. Determine the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.046 g of $C_{25}H_{30}ClNO_5$.

Clemastine Oral Solution

Clemastine Fumarate Oral Solution

Clemastine Oral Solution contains Clemastine Fumarate in a suitable vehicle. Clemastine Oral Solution contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of clemastine, $C_{21}H_{26}ClNO$.

Usual strength. 0.1 mg per ml.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Impurity C. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of a 1.0 per cent w/v solution of *ammonium dihydrogen orthophosphate*.

Test solution. Dilute a quantity of the oral solution containing 0.5 mg of clemastine to 25.0 ml with the solvent mixture.

Reference solution (a). A 0.00008 per cent w/v solution of *clemastine impurity C IPRS (1-(4-chlorophenyl)-1-phenylethanol IPRS)* in the solvent mixture.

Reference solution (b). A solution containing 0.000335 per cent w/v of *clemastine fumarate IPRS* and 0.00008 per cent w/v of *1-(4- chlorophenyl)-1-phenylethanol IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm packed with end-capped octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a mixture of 0.1 volume of *orthophosphoric acid*, 45 volumes of *acetonitrile* and 55 volumes of a 1.0 per cent w/v solution of *ammonium dihydrogen orthophosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Inject reference solution (b).The test is not valid unless the resolution between the peaks due to clemastine fumarate and clemastine impurity C is not less than 2.2.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to clemastine impurity C is not more than the area of the peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. 1 volume of *13.5M ammonia*, 20 volumes of *methanol* and 80 volumes of *stabiliser-free tetrahydrofuran*.

Test solution (a). To a volume of oral solution containing 8 mg of clemastine, add 20 ml of *water*, 20 ml of a saturated

solution of *sodium chloride* and 2.0 ml of *13.5M ammonia*, extract with four 40 ml quantities of *dichloromethane*, washing each extract with the same 40 ml of *water*, filter the dichloromethane extracts and evaporate to dryness at a temperature of 30° to 40° under reduced pressure. Dissolve the residue in 50.0 ml of *methanol*, evaporate to dryness under the same conditions and dissolve the residue in 4 ml of *methanol*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

Reference solution (a). A 0.027 per cent w/v solution of *clemastine fumarate IPRS* in *methanol*.

Reference solution (b). A 0.00135 per cent w/v solution of *clemastine fumarate IPRS* in *methanol*.

Reference solution (c). A solution containing 0.0135 per cent w/v, each of, *clemastine fumarate IPRS* and *diphenhydramine hydrochloride IPRS* in *methanol*.

Reference solution (d). A 0.0054 per cent w/v solution of *2-(2-hydroxyethyl)-1-methylpyrrolidine IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of cold air for 5 minutes. Spray with a freshly prepared mixture of 1 volume of *potassium iodobismuthate solution* and 10 volumes of *2M acetic acid* and then with 10 volumes of *hydrogen peroxide solution*. Cover the plate immediately with a glass plate of the same size and examine the chromatograms after 2 minutes. The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots. In the chromatogram obtained with test solution (a), any spot corresponding to *2-(2-hydroxyethyl)-1-methylpyrrolidine* is not more intense than the spot in the chromatogram obtained with reference solution (d) (2.0 per cent, with reference to clemastine fumarate) and any orange-brown secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent, with reference to clemastine fumarate). Ignore any spot remaining on the line of application and any spot with an R_f value greater than that of the principal spot.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of a 1.0 per cent w/v solution of *ammonium dihydrogen orthophosphate*.

Test solution. Dilute a quantity of the oral solution containing 0.5 mg of clemastine to 20.0 ml with the solvent mixture.

Reference solution. A 0.00335 per cent w/v solution of *clemastine fumarate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a mixture of 0.1 volume of *orthophosphoric acid*, 45 volumes of *acetonitrile* and 55 volumes of a 1.0 per cent w/v solution of *ammonium dihydrogen orthophosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Inject the reference solution and the test solution.

Determinethe weight per ml (2.4.29) of the oral solution and calculate the content of $C_{21}H_{26}ClNO$, weight in volume.

Labelling. The label states the quantity of the active ingredient in terms of the equivalent amount of clemastine.

Clemastine Tablets

Clemastine Fumarate Tablets

Clemastine Tablets contain not less than 93.0 per cent and not more than 105.0 per cent of the stated amount of clemastine, $C_{21}H_{26}ClNO$.

Usual strength. 1 mg.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

B. In the test for Impurity C, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 500 ml of a buffer solution pH 4.0, prepared by dissolving 10 g of *citric acid monohydrate* in 500 ml of *water*, add 11.0 ml of 30 per cent w/v of *sodium hydroxide* and 4.4 ml of *hydrochloric acid* and dilute with *water* to 1000 ml, if necessary adjusted to pH 4.0 with 50 per cent w/v of *sodium hydroxide*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium, centrifuge for 20 minutes at 4000 rpm and filter, Transfer 50.0 ml of the medium to 125-ml separating funnels, add 10 ml of 0.02 per cent w/v of *methyl orange solution*, mix, add 20.0 ml of *chloroform*, shake

mechanically for 10 minutes, remove the chloroform layer and centrifuge the chloroform layer for 10 minute at 4000 rpm. Measure the absorbance at the maximum at about 420 nm. (2.4.7). Calculate the content of $C_{21}H_{26}ClNO,C_4H_4O_4$ in the medium from the absorbance obtained from a solution of known concentration of *clemastine fumarate IPRS* treated in the same manner.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{26}ClNO,C_4H_4O_4$.

Impurity C. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of a 1 per cent w/v solution of *ammonium dihydrogen orthophosphate*.

Test solution. Disperse a quantity of the powdered tablets containing 10 mg of clemastine in 200 ml of the solvent mixture, with the aid of ultrasound for 45 minutes. Centrifuge at 4000 rpm for 10 minutes and use supernatant liquid.

Reference solution (a). A 0.0000335 per cent w/v solution of *clemastine impurity C IPRS (1-(4-chlorophenyl)-1-phenylethanol IPRS)* in the solvent mixture.

Reference solution (b). A 0.0067 per cent w/v solution of *clemastine fumarate IPRS* in the solvent mixture

Reference solution (c). A solution containing 0.000335 per cent w/v of *clemastine fumarate IPRS* and 0.000064 per cent w/v of *clemastine impurity C IPRS* in the solvent mixture.

Use chromatographic system as described under Assay using 100 µl injection volume:

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to clemastine fumarate and clemastine impurity C is not less than 2.2.

Inject referene solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to clemastine impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 1 volume of *13.5 M ammonia*, 20 volumes of *methanol* and 80 volumes of *tetrahydrofuran*.

Test solution (a). Disperse a quantity of the powdered tablets containing 8 mg of clemastine in 4 ml of *methanol* with the aid of ultrasound for 15 minutes. Centrifuge at 4000 rpm for 10 minutes and use supernatant liquid.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol* .

Reference solution (a). A 0.027 per cent w/v solution of *clemastine fumarate IPRS* in *methanol*.

Reference solution (b). A 0.00135 per cent w/v solution of *clemastine fumarate* in *methanol*.

Reference solution (c). A solution containing 0.0135 per cent w/v of *clemastine fumarate IPRS* and *diphenhydramine hydrochloride IPRS* in *methanol*.

Reference solution (d). A 0.00135 per cent w/v solution of *2-(2-hydroxyethyl)-1-methylpyrrolidine IPRS* in *methanol*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in cold air for 5 minutes and spray with a freshly prepared mixture of 1 volume of *potassium iodobismuthate solution* and 10 volumes of *2 M acetic acid* and then with *hydrogen peroxide solution (10 volume)*. Cover the plate immediately with a glass plate of the same size and examine the chromatograms after 2 minutes.

In the chromatogram obtained with test solution (a), any spot corresponding to 2-(2-hydroxyethyl)-1-methylpyrrolidine is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.5 per cent with reference to clemastine fumarate), any orange-brown secondary spot is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent with reference to clemastine fumarate). Ignore any spot remaining on the line of application and any spot with an R_f value more than that of the principal spot. The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay using following solutions.

Test solution. Disperse 1 tablet with 40 ml of the solvent mixture with the aid of ultrasound for 45 minutes, cool and dilute to 50.0 ml with the solvent mixture, centrifuge and use a clear supernatant liquid.

Reference solution. A 0.0027 per cent w/v solution of *clemastine fumarate IPRS* in the solvent mixture.

Calculate the content of $C_{21}H_{26}ClNO$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of a 1 per cent w/v solution of *ammonium dihydrogen orthophosphate*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 10 mg of clemastine in 200 ml of the solvent mixture, with the aid of ultrasound for 45 minutes.Centrifuge at 4000 rpm for 10 minutes and use the supernatant liquid.

Reference solution. A 0.0067 per cent w/v solution of *clemastine fumarate IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.1 volume of *orthophosphoric acid*, 50 volumes of *acetonitrile* and 50 volumes of a 1.0 per cent w/v solution of *ammonium dihydrogen orthophosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

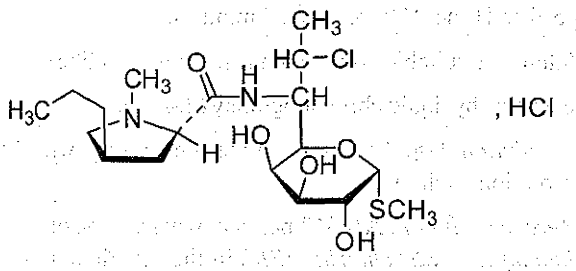
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{26}ClNO$ in the tablets.

Labelling. The quantity of the active ingredient is stated in terms of the equivalent amount of clemastine.

Clindamycin Hydrochloride



$C_{18}H_{33}ClN_2O_5S,HCl$ Mol. Wt. 461.5

Clindamycin Hydrochloride is methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propyl-2-pyrrolidinyl] carbonyl] amino]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside hydrochloride.

Clindamycin Hydrochloride contains not less than 91.0 per cent and not more than 102.0 per cent of $C_{18}H_{33}ClN_2O_5S,HCl$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *clindamycin hydrochloride IPRS* or with the reference spectrum of clindamycin hydrochloride.

B. Determine by thin- layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 19 volumes of *2-propanol*, 38 volumes of a 15 per cent w/v solution of *ammonium acetate*, adjusted to pH 9.6 with *ammonia* and 43 volumes of *ethyl acetate*.

Test solution. Dissolve 10 mg of the substance under examination in 10.0 ml of *methanol*.

Reference solution (a). A 0.1 per cent w/v solution of *clindamycin hydrochloride IPRS* in the *methanol*.

Reference solution (b). A solution containing 0.1 per cent w/v, each of, *clindamycin hydrochloride IPRS* and *lincomycin hydrochloride IPRS* in the *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the pate in air, spray with a 0.1 per cent w/v solution of *potassium permanganate*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

C. Dissolve about 10 mg of the substance under examination in 2 ml of *dilute hydrochloric acid* and heat on a water-bath for 3 minutes, add 3 ml of *sodium carbonate solution* and 1 ml of a 2 per cent w/v solution of *sodium nitroprusside*, a violet-red colour is produced.

D. A 1 per cent w/v solution gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 3.0 to 5.0, determined in a 10 per cent w/v solution in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). +135° to +150°, determined in a 4.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *clindamycin hydrochloride IPRS* in the mobile phase.

Reference solution (b). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of a 0.68 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 7.5 with a 25 per cent w/v solution of *potassium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

The relative retention time with reference to clindamycin for methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-galacto-octopyranoside (clindamycin impurity A) is about 0.4; for methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-4-ethyl-1-methylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-*threo*-α-D-galacto-octopyranoside (clindamycin impurity B) is about 0.65 and for methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-galacto- octopyranoside (clindamycin impurity C) is about 0.8.

Inject reference solution (b) and the test solution. In the chromatogram obtained with test solution the area of the peak due to clindamycin impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). The area of peak corresponding to clindamycin impurity C is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent). Ignore any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). 3.0 per cent to 6.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{33}ClN_2O_5S$,HCl.

Storage. Store protected from moisture.

Clindamycin Capsules

Clindamycin Hydrochloride Capsules

Clindmycin Capsules contain Clindamycin Hydrochloride equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clindamycin, $C_{18}H_{33}ClN_2O_5S$.

Usual strength. 150 mg.

Identification

A. Shake a quantity of the content of capsules containing about 30 mg of clindamycin with 15 ml of *chloroform*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clindamycin hydrochloride IPRS* or with the reference spectrum of clindamycin hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.017 per cent w/v solution of *clindamycin hydrochloride IPRS* in the dissolution medium.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: Dissolve 4 g of *d-10-camphorsulfonic acid*, 2 g of *ammonium acetate*, and 2 ml of *glacial acetic acid* in 400 ml of *water* and dilute to 1000.0 ml with *methanol*, adjusted to pH 6.0 with *hydrochloric acid* or *5 M sodium hydroxide*,
- flow rate: 2 ml per minute,
- refractive index detector,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the medium.

Q. Not less than 80 per cent of the stated amount of clindamycin $C_{18}H_{33}ClN_2O_5S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the content of capsules containing about 50 mg of clindamycin with 50 ml of the mobile phase for 15 minutes and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Reference solution (b). A 0.1 per cent w/v solution of *clindamycin hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of 0.68 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 7.5 with 25 per cent w/v solution of *potassium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (b). The relative retention time with reference to clindamycin for methyl 6,8-dideoxy-6-[[[(2*S*,4*R*)-1- methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-erythro-α-D-galacto- octopyranoside (lincomycin) (clindamycin impurity A) is about 0.4, for methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-4-ethyl- 1-methylpyrrolidin-2-yl]carbonyl]amino]-1-thio-*L-threo*-α-D-*galacto*-octopyranoside (clindamycin B) (clindamycin impurity B) is about 0.65 and for methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*- octopyranoside (7-epiclindamycin) (clindamycin impurity C) is about 0.8.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of peak corresponding to clindamycin impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent), the area of peak corresponding to clindamycin impurity C is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (6.0 per cent). Ignore any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Capsules.

Water (2.3.43). Not more than 7.0 per cent, determined on 1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the content of 20 capsules containing about 50 mg of clindamycin with 50 ml of the mobile phase for 15 minutes and filter.

Reference solution. A 0.11 per cent w/v solution of *clindamycin hydrochloride IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

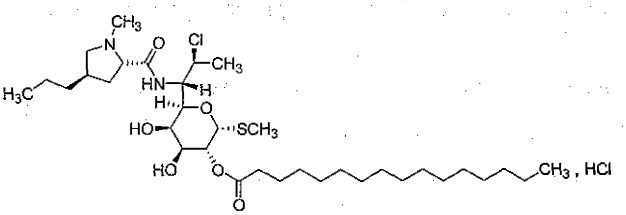
Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the capsules.

1 mg of $C_{18}H_{33}ClN_2O_5S$, HCl is equivalent to 0.9209 mg of $C_{18}H_{33}ClN_2O_5S$.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of clindamycin.

Clindamycin Palmitate Hydrochloride



$C_{34}H_{63}ClN_2O_6S,HCl$ Mol. Wt. 699.9

Clindamycin Palmitate Hydrochloride is L-*threo*-α-D-*galacto*-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-2-hexadecanoate, monohydrochloride, (2*S-trans*)-;Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L *threo*-α-D-*galacto*-octopyranoside 2-palmitate monohydrochloride.

Clindamycin Palmitate Hydrochloride has a potency equivalent to not less than 540 µg of clindamycin per mg.

Category. Antibiotics.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clindamycin palmitate hydrochloride IPRS* or with the reference spectrum of clindamycin palmitate hydrochloride.

Tests

pH (2.4.24). 2.8 to 3.8, determined in a 1.0 per cent w/v solution.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). Not more than 3.0 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.35 g of the substances under examination in the mobile phase and dilute to 25.0 ml with the same solvent.

Reference solution. A 1.4 per cent w/v solution of *clindamycin palmitate hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase. Dissolve 2 g of *docusate sodium* and 1.54 g of *ammonium acetate* in a mixture of 2 ml of *glacial acetic acid* and 75 ml of *water* and dilute with *methanol* to 1000 ml and pass through a suitable filter and degas,
- flow rate: 1.2 ml per minute,
- refractive index detector,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and test solution.

Calculate the content of $C_{34}H_{63}ClN_2O_6S$.

Storage. Store protected from moisture

Clindamycin Palmitate Hydrochloride Oral Suspension

Clindamycin Palmitate Hydrochloride Oral Solution is a mixture consisting of clindamycin palmitate hydrochloride and one or more suitable buffers, colours, diluents, flavours, and preservatives. It is filled in sealed containers. The oral solution is constituted by dispersing the contents of the sealed container in the specified volume of *water* just before use.

Clindamycin Palmitate Hydrochloride Oral Solution contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of clindamycin, $C_{18}H_{33}ClN_2O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted solution may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of clindamycin $C_{18}H_{33}ClN_2O_5S$.

Usual strength. 15 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

The constituted solution complies with the tests stated under oral liquids and with the following tests.

Tests

pH (2.4.24). 2.5 to 5.0, in the solution constituted as directed in the labelling.

Water (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

Other tests. Comply with the tests stated under Oral Powders.

Assay. Determine by gas chromatography (2.4.13).

Solution A. A 30.0 per cent w/v of *sodium carbonate*.

Internal standard solution. A. 0.5 per cent w/v of cholesteryl benzoate in *chloroform*.

Test solution. Transfer 5 ml of the constituted solution to a centrifuge tube. Add 5.0 ml of internal standard solution and 1 ml of solution A. Insert the stopper, shake vigorously for 10 minutes and centrifuge. Remove the upper aqueous layer, and transfer 1.0 ml of the lower chloroform layer to a centrifuge tube. Add 1.0 ml of *pyridine* and 1.0 ml of *acetic anhydride*. Agitate the tube to ensure complete mixing, cover the top of the centrifuge tube with a plastic cap through which a small hole has been punched, heat at 100° for 2.5 hours, and allow to cool, Mix and centrifuge, if necessary.

Reference solution. Transfer 150 mg of *clindamycin palmitate hydrochloride IPRS* to a centrifuge tube. Add 5 ml of *water*, 5.0 ml of internal standard solution, and 1 ml of solution A. Insert the stopper, shake vigorously for 10 minutes and centrifuge. Remove the upper aqueous layer and transfer 1.0 ml of the lower chloroform layer to a centrifuge tube. Add 1.0 ml of *pyridine* and 1.0 ml of *acetic anhydride*. Agitate the tube to ensure complete mixing, cover the top of the centrifuge tube with a plastic cap through which a small hole has been punched, heat at 100° for 2.5 hours and allow to cool. Mix and centrifuge, if necessary.

Chromatographic system

- a glass column 0.6 m x 3.0 mm packed with 1 per cent vinyl 5 per cent phenylmethylpolysiloxane,
- temperature column: 290°,

- flame ionization detector at 320°,
- flow rate: 60 ml per minute, using nitrogen as the carrier gas,
- injection volume: 1 µl.

Inject the reference solution. The elution order of peaks is cholesteryl benzoate and clindamycin palmitate.

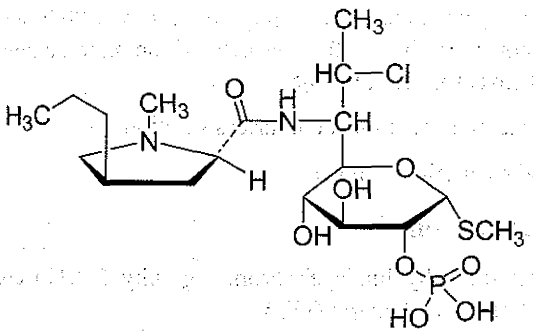
Inject the reference solution and the test solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of clindamycin, $C_{18}H_{33}ClN_2O_5S$, weight in volume.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. (1) The label states the strength in terms of the equivalent amount of clindamycin; (2) The temperature of storage and the period during which the constituted oral liquids may be expected to be satisfactory for use.

Clindamycin Phosphate



$C_{18}H_{34}ClN_2O_8PS$ Mol. Wt. 505.0

Clindamycin Phosphate is methyl 7-chloro-6,7,8-trideoxy-6-[[[(2*S*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-threo-α-D-galacto-octopyranoside 2-(dihydrogen phosphate). It is a semi-synthetic product derived from a fermentation product.

Clindamycin Phosphate contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{18}H_{34}ClN_2O_8PS$, calculated on the anhydrous basis.

Category. Lincosamide antibacterial.

Description. A white or almost white, slightly hygroscopic powder. It shows polymorphism (2.5.11).

Identification

Tests B, C and D may be omitted if tests A and D are carried out. Tests A and D may be omitted if tests B, C and D are carried out.

A. In 2 separate tubes place 50 mg of the substance under examination and 50 mg of *clindamycin phosphate IPRS*. Add 0.2 ml of *water* and heat until completely dissolved. Evaporate to dryness under reduced pressure and dry the residues at 100° to 105° for 2 hours. On the residue, determine by infrared absorption spectrophotometry (2.4.6), compare the spectrum with that obtained with *clindamycin phosphate IPRS* or with the reference spectrum of clindamycin phosphate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 20 volumes of *glacial acetic acid*, 20 volumes of *water* and 60 volumes of *butanol*.

Test solution. Dissolve 20 mg of the substance under examination in *methanol* and dilute to 10 ml with *methanol*.

Reference solution (a). A 0.2 per cent w/v solution of *clindamycin phosphate IPRS* in *methanol*.

Reference solution (b). Dissolve 10 mg of *lincomycin hydrochloride IPRS* in 5 ml of reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 100 to 105° for 30 minutes and spray with a 0.1 per cent w/v solution of *potassium permanganate*. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The chromatogram obtained with reference solution (b) shows 2 principal spots.

C. Dissolve 10 mg in 2 ml of *dilute hydrochloric acid* and heat in a water-bath for 3 minutes. Add 4 ml of *sodium carbonate solution* and 1 ml of a 2.0 per cent w/v solution of *sodium nitroprusside*. Prepare a reference solution in the same manner using *clindamycin phosphate IPRS*. The colour of the test solution corresponds to that of the reference solution.

D. Boil 0.1 g under a reflux condenser with a mixture of 5 ml of *strong sodium hydroxidesolution* and 5 ml of *water* for 90 minutes. Cool and add 5 ml of *nitric acid*. Extract with 3 quantities, each of 15-ml, of *dichloromethane* and discard the extracts. Filter the upper layer through a paper filter. The filtrate gives reaction (b) of phosphates (2.3.1).

Tests

Solution A. Dissolve 1.0 g in *carbon dioxide-free water*. Heat gently if necessary. Cool and dilute to 25.0 ml with *carbon dioxide-free water*.

Appearance of the solution. Solution A is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 3.5 to 4.5, determined by diluting 5.0 ml of solution A to 20 ml with *carbon dioxide-free water*.

Specific optical rotation (2.4.22). + 115.0° to + 130.0°, determined on 1.0 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 75 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). A 0.3 per cent w/v solution of *clindamycin phosphate IPRS* in the mobile phase.

Reference solution (b). Dissolve 5 mg of *clindamycin impurity A IPRS (lincomycin hydrochloride IPRS)* and 15.0 mg of *clindamycin impurity E IPRS (clindamycin hydrochloride IPRS)* in 5.0 ml of reference solution (a) and then dilute to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 200 volumes of *acetonitrile* and 800 volumes of a 1.36 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to clindamycin phosphate (2nd peak) and clindamycin impurity E (3rd peak) is not less than 6.0, the tailing factor for the peak due to clindamycin phosphate is not more than 1.5. The peak due to clindamycin impurity A (1st peak) is clearly separated from the peak due to the solvent.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak due to clindamycin phosphate in the chromatogram obtained with reference solution (c) (2.5 per cent) and the sum of the areas of all the secondary peaks is not more than 4 times the area of the peak due to clindamycin phosphate in the chromatogram obtained with reference solution (c) (4.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Water (2.3.43). Not more than 6.0 per cent, determined on 0.25 g.

Assay. Determine by liquid chromatography (2.4.14) as described under test for Related substances with the following modifications.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{34}ClN_2O_8PS$.

Clindamycin Phosphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.6 Endotoxin Unit per mg.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Clindamycin Injection

Clindamycin Phosphate Injection

Clindamycin Injection is a sterile solution of Clindamycin Phosphate in Water for Injections.

Clindamycin Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of clindamycin, $C_{18}H_{33}ClN_2O_5S$.

Description. An almost colourless solution.

Usual strength. 150 mg per ml.

Identification

A. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1.5 volumes of *18 M ammonia*, 30 volumes of *toluene* and 70 volumes of *methanol*.

Test solution. Dilute a volume of the injection containing 50 mg of Clindamycin to 10 ml with *methanol*.

Reference solution. A 0.5 per cent w/v solution of *clindamycin phosphate IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 5.5 to 7.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection with the mobile phase to obtain a solution containing 0.3 per cent w/v of Clindamycin.

Reference solution. A solution containing 0.012 per cent w/v, each of, *lincomycin hydrochloride IPRS*, 0.024 per cent w/v of *clindamycin phosphate IPRS* and 0.0015 per cent v/v of *benzyl alcohol* in the mobile phase.

Use chromatographic system as described under Assay.

The order of elution is lincomycin phosphate, clindamycin phosphate and benzyl alcohol.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to lincomycin hydrochloride and clindamycin phosphate is not less than 7.7.

Inject the test solution. The sum of the areas of all the secondary peaks is not more than 8.0 per cent, calculated by area normalization. Ignore any peak obtained due to benzyl alcohol.

Bacterial endotoxins (2.2.3). Dilute the injection in *water BET* to give a solution containing 10 mg per ml. The solution contains not more than 6.0 Endotoxin Units per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Dilute a volume of the injection with the mobile phase to obtain a solution containing 0.015 per cent w/v of Clindamycin.

Reference solution (a). A 0.018 per cent w/v solution of *clindamycin phosphate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.012 per cent w/v of *lincomycin hydrochloride IPRS*, 0.024 per cent w/v of *clindamycin phosphate IPRS* and 0.0015 per cent v/v of *benzyl alcohol* in the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes 1.36 per cent w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to lincomycin hydrochloride and clindamycin phosphate is not less than 7.7.

Inject reference solution (a) and the test solution.

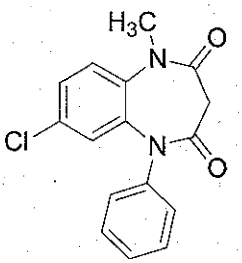
Calculate the content of $C_{18}H_{33}ClN_2O_5S$ in the injection.

1 mg of $C_{18}H_{34}ClN_2O_8PS$ is equivalent to 0.8416 mg of $C_{18}H_{33}ClN_2O_5S$.

Storage. Store at a temperature not exceeding 30°. The injection should not be refrigerated and it should not be allowed to freeze.

Labelling. The label states the strength in terms of the equivalent amount of Clindamycin in a suitable dose volume.

Clobazam



$C_{16}H_{13}ClN_2O_2$ Mol. Wt. 300.7

Clobazam is 7-chloro-1-methyl-5-phenyl-1,5-dihydro-3*H*-1,5-benzodiazepine-2,4-dione.

Clobazam contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{16}H_{13}ClN_2O_2$, calculated on the dried basis.

Category. Anticonvulsant.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clobazam IPRS* or with the reference spectrum of clobazam.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 50 ml with the mobile phase.

Reference solution (a). Dissolve 5.0 mg of *7-chloro-5-phenyl-1,5-dihydro-3H-1,5-benzodiazepine-2,4-dione IPRS (clobazam impurity A)* in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg, each of, *chlordiazepoxide IPRS* and *clonazepam IPRS* in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (b). The resolution between the peaks due to chlordiazepoxide and clonazepam is not less than 1.3.

Inject the test solution and reference solution (a) and (c). Continue the chromatography for 5 times the retention time of clobazam (about 15 minutes). In the chromatogram obtained with the test solution the area of the peak obtained due to impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other impurity peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent) and the sum of the areas of all other impurity peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on the residue obtained in the test for Loss on drying.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° to 105°.

Assay. Weigh accurately about 50 mg and dissolve in 100.0 ml of *ethanol (95 per cent)*. Dilute 2.0 ml of the solution to 250.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7), taking 1380 as the specific absorbance at 232 nm.

Calculate the content of $C_{16}H_{13}ClN_2O_2$.

Storage. Store protected from moisture.

Clobazam Tablets

Clobazam Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clobazam, $C_{16}H_{13}ClN_2O_2$.

Usual strengths. 5mg; 10 mg; 20 mg.

Identification

A. Shake a quantity of the powdered tablets containing 20 mg of Clobazam with 10 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. Dissolve the residue in the minimum amount of *methanol*, evaporate to dryness and dry the residue at 105° for 10 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clobazam IPRS* or with the reference spectrum of clobazam.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.1M hydrochloric acid*,
Speed and time: 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute with equal volumes of *acetonitrile* and *water*, if necessary, to produce a solution expected to contain 0.0005 per cent w/v of clobazam.

Reference solution. A 0.0005 per cent w/v solution of *clobazam IPRS* in a mixture of equal volumes of *acetonitrile* and *water*.

Use the chromatographic system as described under Related substances, using 50 µl injection volume.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{13}ClN_2O_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{13}ClN_2O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 25 mg of Clobazam in 25 ml of mobile phase, mix with the aid of ultrasound, dilute to 50.0 ml with the mobile phase centrifuge and use the supernatant liquid.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase and further dilute 1.0 ml the solution to 10.0 ml with the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *7-chloro-1,5-dihydro-5-phenyl-1, 5-benzodiazepine-2,4(3H)-dione IPRS* (*clobazam impurity A IPRS*) in *methanol*. Dilute 2.5 ml of the solution to100.0 ml with the mobile phase.

Reference solution (c). A 0.01 per cent w/v solution of *7-chloro-1,5-dihydro-5-phenyl-1,5- benzodiazepine-2,4(3H)-*

dione IPRS (clobazam impurity A IPRS) in *methanol*. Dilute 1.0 ml of the solution to 2.0 ml with a 0.1 per cent w/v solution of *clobazam IPRS* in the mobile phase.

Reference solution (d). Dilute 1.0 ml of reference solution (a) to 2.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 15 cm x 2.0 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm) (Such as Nucleosil C18),
- column temperature: 40°,
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*,
- flow rate: 0.25 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 25 µl.

Inject reference solution (c). The test is not valid unless, the resolution between the peaks due to clobazam impurity A and clobazam is not less than 3.0.

Inject reference solution (a), (b), (d) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to clobazam impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay with the following test solution.

Test solution. Transfer one tablet in a 50.0 ml volumetric flask, add 3 ml of *water* and allow the tablet to disperse with the aid of ultrasound. Add 30 ml of mobile phase and mix with the aid of ultrasound for 10 minutes, dilute to 50.0 ml with mobile phase and filter. Dilute the filtrate with mobile phase to obtain a solution containing 0.002 per cent of clobazam.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances with the following modifications.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Clobazam in 80 ml of mobile phase, mix with the aid of ultrasound, dilute to 100.0 with mobile phase and centrifuge. Dilute 1.0 ml of the supernatant liquid to 10.0 ml with mobile phase.

Reference solution (a). A 0.002 per cent w/v solution of *clobazam IPRS* in mobile phase.

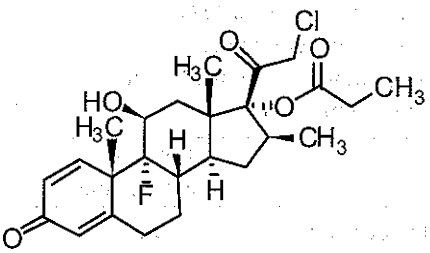
Reference solution (b). A 0.01 per cent w/v solution of *7-chloro-1,5-dihydro-5-phenyl-1,5- benzodiazepine-2,4(3H)-dione IPRS* (*clobazam impurity A IPRS*) in mobile phase. Dilute 1.0 ml of the solution to 2.0 ml with a 0.1 per cent w/v solution of *clobazam IPRS* in the mobile phase.

Inject reference solution (b). The test is not valid unless, the resolutionbetween clobazam impurity A and clobazam is not less than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{13}ClN_2O_2$ in the tablets.

Clobetasol Propionate



$C_{25}H_{32}ClFO_5$ Mol. Wt. 467.0

Clobetasol Propionate is 21-chloro-9α-fluoro-11b-hydroxy-16β-methylpregna-1,4-diene-3,20-dion-17α–yl propionate..

Clobetaol propionate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{25}H_{32}ClFO_5$, calculated on the dried basis.

Category. Glucocorticoid.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clobetasol propionate IPRS* or with the reference spectrum of clobetasol propionate.

Tests

Specific optical rotation (2.4.22). + 112° to + 118°, determined in a 1.0 per cent w/v solution in *acetone*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 20.0 ml with the mobile phase.

Test solution (b). Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve the contents of a vial of *clobetasol impurity J IPRS* in 2.0 ml of the mobile phase. To 0.5 ml of the solution, add 0.5 ml of test solution (b) and dilute to 20.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 50.0 ml with the mobile phase. Further dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *methanol*, 42.5 volumes of a 0.785 per cent w/v solution of *sodium dihydrogen phosphate monohydrate,* adjusted to pH 5.5 with 10 per cent w/v of *sodium hydroxide* and 47.5 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Clobetasol impurity A[1]	0.4	—
Clobetasol impurity B[2]	0.6	0.6
Clobetasol impurity C[3]	0.9	1.5
Clobetasol	1.0	—
Clobetasol impurity J[4]	1.1	—
Clobetasol impurity D[5]	1.2	—
Clobetasol impurity L[6]	1.3	—
Clobetasol impurity M[7]	1.6	—
Clobetasol impurity E[8]	2.1	—

[1]betamethasone 17-propionate,

[2]21-chloro-9-fluoro-11β-hydroxy-16-methylpregna-1,4,16-triene-3,20-dione,

[3]21-chloro-9-fluoro-11b-hydroxy-16a-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate,

[4](17R)-4'-chloro-5'-ethyl-9-fluoro-11b-hydroxy-16b-methylspiro[androsta-1,4-diene-17,2'(3'H)-furan]-3,3'-dione,

[5]1,2-dihydroclobetasol 17-propionate,

[6]unknown structure,

[7]unknown structure,

[8]21-chloro-16b-methyl-3,20-dioxopregna-1,4-dien-17-yl propanoate.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to clobetasol propionate and clobetasol propionate impurity J is not less than 2.0.

Inject reference solution (b) and test solution (a). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution (a), the area of any peak due to clobetasol impurity E is not more than 1.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent). The area of any peak due to clobetasol impurity D is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak due to clobetasol impurities B and C is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any peak due to clobetasol impurities A, L and M is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances using following solutions.

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *clobetasol propionate IPRS* in the mobile phase.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{32}ClFO_5$.

Storage. Store protected from light.

Clobetasol Cream

Clobetasol Propionate Cream

Clobetasol Cream contains Clobetasol Propionate in a suitable cream base.

Clobetasol Cream contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of clobetasol propionate, $C_{25}H_{32}ClFO_5$.

Usual strength. 0.05 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 5 volumes of *ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane*.

Test solution. Transfer a quantity of the cream containing 0.75 mg of Clobetasol Propionate to a 25-ml centrifuge tube, add 10 ml of *methanol* and heat in a water-bath at 60° for 4 minutes. Remove from the water-bath and shake vigorously. Repeat the heating and shaking, cool to room temperature, add 3.5 ml of *water* and mix. Centrifuge for 10 minutes. Transfer 10 ml of the clear supernatant liquid to a 100-ml separating funnel, add 1 g of *sodium chloride* and 10 ml of *water* and mix. Add 5 ml of *dichloromethane* and shake for 1 minute. Evaporate the dichloromethane layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 0.5 ml of *dichloromethane*.

Reference solution (a). A 0.05 per cent w/v solution of *clobetasol propionate IPRS* in *dichloromethane*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 10 µl of each solution. After removal of the plate, dry in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (a) corresponds to the peak in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

CAUTION — Prepare the test solutions with full facial protection and wearing heat-resistant gloves.

Test solution (a). Disperse a quantity of the cream containing 1 mg of Clobetasol Propionate in 10 ml of *ethanol*, stopper firmly using a plastic stopper, heat on a water-bath with intermittent shaking until the cream is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 10 ml with *ethanol*.

Test solution (b). Prepare in the same manner as test solution (a), but add 5 ml of a 0.04 per cent w/v solution of *beclometasone dipropionate IPRS* in *ethanol* and 5 ml of *ethanol*.

Reference solution. A solution containing 0.005 per cent w/v of *clobetasol propionate IPRS* and 0.01 per cent w/v of *beclometasone dipropionate IPRS* (internal standard) in *ethanol*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 60°,
- mobile phase: a mixture of 45 volumes of *ethanol* and 55 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution, test solution (a) and (b).

Calculate the content of $C_{25}H_{32}ClFO_5$ in the cream.

Storage. Store at a temperature not exceeding 30°.

Clobetasol Ointment

Clobetasol Propionate Ointment

Clobetasol Ointment contains Clobetasol Propionate in a suitable base.

Clobetasol Ointment contains not less than 90.0 per cent and not more than 115.0 per cent w/w of clobetasol propionate, $C_{25}H_{32}ClFO_5$.

Usual strength. 0.05 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 5 volumes of *ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane*.

Test solution. Disperse a quantity of ointment containing 0.5 mg of Clobetasol Propionate to a 25-ml centrifuge tube, add 10 ml of *methanol* and heat in a water-bath at 70° for 4 minutes. Remove from the water- bath and shake vigorously. Repeat the heating and shaking, cool in ice for 5 minutes and centrifuge for 10 minutes. Transfer 5 ml of the clear supernatant liquid to a suitable vial, evaporate to dryness in a current of nitrogen and dissolve the residue in 0.5 ml of *dichloromethane*.

Reference solution (a). A 0.05 per cent w/v solution of *clobetasol propionate IPRS* in *dichloromethane*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 10 µl of each solution. After removal of the plate, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Other tests. Comply with the tests stated under Ointments.

Assay. Determine by liquid chromatography (2.4.14).

CAUTION—Prepare the solutions with full facial protection and wearing heat-resistant gloves.

Test solution. Disperse a quantity of the ointment containing 1 mg of clobetasol Propionate with 10 ml of *ethanol*, stopper firmly using a plastic stopper, heat on a water-bath with intermittent shaking. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 10 ml with *ethanol*.

Reference solution (a). A solution containing 0.005 per cent w/v of *clobetasol propionate IPRS* and 0.01 per cent w/v of *beclometasone dipropionate IPRS* (internal standard) in *ethanol*.

Reference solution (b). Disperse a quantity of the ointment containing1 mg of clobetasol Propionate with 5 ml of 0.04 per cent w/v solution of *beclometasone dipropionate IPRS* in *ethanol* and 5 ml of *ethanol*, heat on a water-bath with intermittent shaking. Cool the contents in ice for 30 minutes, centrifuge and dilute 5 ml of the supernatant liquid to 10 ml with *ethanol*.

Chromatographic system

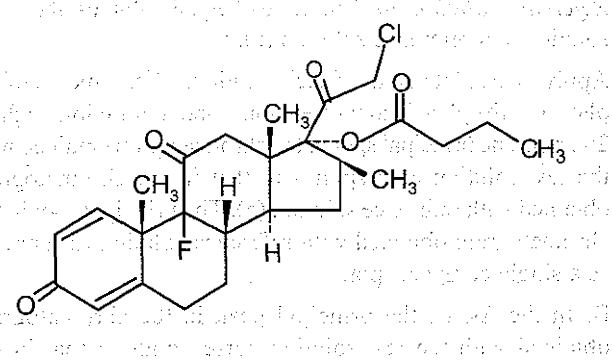
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 60°,
- mobile phase: a mixture of 45 volumes of *ethanol* and 55 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (a), (b) and the test solution.

Calculate the content of $C_{25}H_{32}ClFO_5$ in the ointment.

Storage. Store at a temperature not exceeding 30°.

Clobetasone Butyrate



$C_{26}H_{32}ClFO_5$ Mol. Wt. 479.0

Clobetasone Butyrate is (16β)-21-chloro-9-fluoro-16-methylpregna-1,4-dien—3,11,20-trione-17-butyrate.

Clobetasone Butyrate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{26}H_{32}ClFO_5$, calculated on the dried basis.

Category. Glucocorticoid.

Description. A white to off-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clobetasone butyrate IPRS* or with the reference spectrum of clobetasone butyrate.

Tests

Specific optical rotation (2.4.22). + 131.0° to + 138.0°, determined in a 1.0 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Prepare the solutions immediately before use.

Solvent mixture. A mixture of 0.1 volume of *anhydrous formic acid*, 43 volumes of *acetonitrile* and 57 volumes of *water*.

Test solution. Dissolve 65 mg of the substance under examination in 5 ml of *acetonitrile* and dilute to 25.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 0.1 volume of *anhydrous formic acid* and 99.9 volumes of *water*,
  B: a mixture of 0.1 volume of *anhydrous formic acid* and 99.9 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 241 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cen v/v)	Mobile phase B (per cent v/v)
0	57	43
3	57	43
26	43	57
30	57	43

The relative retention time with reference to clobetasone butyrate (Retention time: about 14 minutes) for clobetasone impurity F (16α-methyl clobetasone butyrate) is about 0.9.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 20 mg in *ethanol (95 per cent)* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *ethanol (95 per cent)*. Measure the absorbance at the absorption maximum at 235 nm (2.4.7). Calculate the content of $C_{26}H_{32}ClFO_5$, taking 327 as the specific absorbance at 235 nm.

Storage. Store protected from light.

Clobetasone Cream

Clobetasone Butyrate Cream

Clobetasone Cream contains clobetasone butyrate in a suitable cream base.

Clobetasone Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clobetasone butyrate, $C_{26}H_{32}ClFO_5$.

Usual strength. 0.05 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 5 volumes of *ethanol*, 10 volumes of *acetone* and 100 volumes of *chloroform*.

Test solution. Disperse a quantity of the cream containing 0.5 mg of Clobetasone Butyrate in a mixture of 5 volumes of *ethanol (80 per cent)* and 10 volumes of *n-hexane*, taking 15 ml of the solvent mixture for each g of cream. Shake the mixture, allow to separate, filter the aqueous layer and add 1 ml of *water* for every 10 ml of *n-hexane* used. Cool the solution in ice for 30 minutes, centrifuge, filter the supernatant liquid and dilute with 10 ml of *water* for every 10 ml of *n-hexane* used. Add 1 g of *sodium chloride* for every 10 ml of *water* used and extract with 5 ml of *chloroform* for every 10 ml of *water* used. Evaporate the chloroform layer to dryness in a current of dry nitrogen with gentle heating and dissolve the residue in 0.5 ml of *chloroform*.

Reference solution (a). A 0.1 per cent w/v of *clobetasone butyrate IPRS* in *chloroform*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 10 µl of each solution. After removal of the plate, allow it to dry in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

B. In the Assay, the chromatogram obtained with test solution (a) corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

CAUTION — Prepare the test solutions with full facial protection and wearing heat-resistant gloves.

Test solution (a). Add 10 ml of *ethanol* to a quantity of the cream containing 1 mg of Clobetasone Butyrate. Stopper firmly using a plastic stopper and heat on a water-bath with intermittent shaking until the cream is completely dispersed. Cool the contents in ice for 30 minutes, centrifuge. Dilute 5 ml of the supernatant liquid to 10 ml with *ethanol*.

Test solution (b). Prepare in the same manner as test solution (a) but adding 5 ml of *ethanol* and 5 ml of a 0.014 per cent w/v solution of the internal standard in *ethanol*.

Reference solution. A solution containing 0.005 per cent w/v of *clobetasone butyrate IPRS* and 0.0035 per cent w/v of *clobetasol propionate IPRS* (internal standard) in *ethanol*.

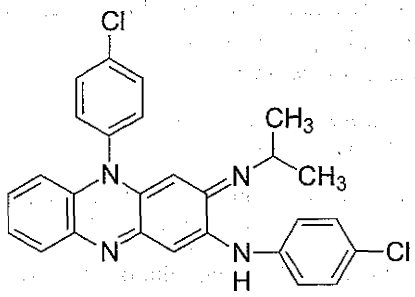
Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 60°,
- mobile phase: a mixture of 40 volumes of *ethanol* and 60 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 241 nm,
- injection volume: 20 µl.

Calculate the content of $C_{26}H_{32}ClFO_5$ in the cream.

Storage. Store at a temperature not exceeding 30°.

Clofazimine



$C_{27}H_{22}Cl_2N_4$ Mol. Wt. 473.4

Clofazimine is 3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine.

Clofazimine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{27}H_{22}Cl_2N_4$, calculated on the dried basis.

Category. Antibacterial (antileprotic).

Description. Dark red crystals or a reddish-brown, fine powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clofazimine IPRS* or with the reference spectrum of clofazimine.

B. When examined in the range 230 nm to 600 nm, a 0.0005 per cent w/v solution in *0.01 M methanolic hydrochloric acid* shows absorption maxima, at about 283 nm and 487 nm; absorbance at about 283 nm, about 0.65 and at about 487 nm, about 0.32 (2.4.7).

C. Dissolve 2 mg in 3 ml of *acetone* and add 0.1 ml of *hydrochloric acid;* an intense violet colour is produced. Add 0.5 ml of *5 M sodium hydroxide*; the colour changes to orange-red.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v, each of, *clofazimine IPRS* and *iminophenazine IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of buffer solution prepared by dissolving 2.25 g of *sodium lauryl sulphate*, 0.85 g of *tetrabutylammonium hydrogen sulphate* and 0.885 g of *disodium hydrogen phosphate* in *water*, adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 500 ml with *water* and 65 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time
Clofazimine impurity A[1]	0.7
Clofazimine impurity B[2]	0.8
Clofazimine (Retention time: about 15 minutes)	1.0

[1] (*N*,5-bis(4-chlorophenyl)-3-imino-3,5-dihydrophenazin-2-amine),

[2](5-(4-chlorophenyl)-3-[(1-methylethyl)imino]-*N*-phenyl-3,5-dihydrophenazin-2-amine).

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 2.0 and the column efficiency of the peak due to clofazimine is not less than 3000 theoretical plates.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to clofazimine impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the area of peak corresponding to clofazimine impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g and dissolve in 20 ml of *chloroform*. Add 50 ml of *acetone* and titrate with *0.1 M perchloric acid* in *dioxan*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04734 g of $C_{27}H_{22}Cl_2N_4$.

Clofazimine Capsules

Clofazimine Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clofazimine, $C_{27}H_{22}Cl_2N_4$.

Usual strength. 100 mg.

Identification

A. When examined in the range 260 nm to 600 nm (2.4.7), a 0.0005 per cent w/v solution in a mixture prepared by dissolving 2.25 g of *sodium dodecyl sulphate*, 0.85 g of *tertbutylammonium hydrogen sulphate* and 0.885 g of *disodium hydrogen orthophosphate* in 500 ml of *water*, adjusted to pH 3.0 with *orthophophoric acid* and 65 volumes of *acetonitrile*, shows two absorbance maximum at 289 nm and 491 nm.

B. To 5 mg of the contents of a capsule add 3 ml of *chloroform* and 1 ml of *2 M hydrochloric acid*; the colour of the chloroform layer changes to violet. Add 2 ml of *2 M sodium hydroxide*; the colour changes to brownish-yellow.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the contents of capsules containing 0.5 g of Clofazimine in the mobile phase and dilute to 100.0 ml with the mobile phase and filter. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (a). A 0.0000125 per cent w/v solution of *iminophenazine IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v, each of, *clofazimine IPRS* and *iminophenazine IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Nucleosil C8),
- mobile phase: a mixture of 35 volumes of buffer solution prepared by dissolving 2.25 g of *sodium dodecyl sulphate*, 0.85 g of *tetrabutylammonium hydrogen sulphate* and 0.885 g of *disodium hydrogen orthophosphate* in 500 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 65 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time
Iminophenazine	0.7
Clofazimine (Retention time: about 15 minutes)	1.0

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 2.0 and the column efficiency of the peak due to clofazimine is not less than 3000 theoretical plates.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

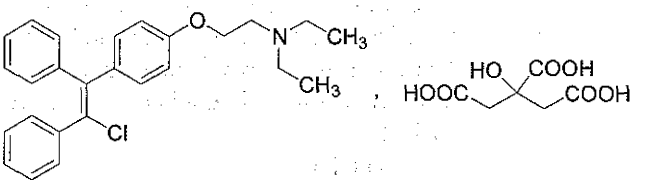
Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.15 g of Clofazimine and dissolve in sufficient *chloroform* to produce 100.0 ml. Filter through a chloroform-washed plug of cotton wool. Dilute 5.0 ml of the clear filtrate to 100.0 ml with *chloroform*. To 5.0 ml add 5.0 ml of *0.1 M methanolic hydrochloric acid* and sufficient *chloroform* to produce 50.0 ml. Measure the *absorbance* of the resulting solution at the maximum at about 491 nm (2.4.7), using as the blank a mixture of 5.0 ml of *0.1 M methanolic hydrochloric acid* and sufficient *chloroform* to produce 50.0 ml. Calculate the content of $C_{27}H_{22}Cl_2N_4$ taking 650 as the specific absorbance at 491 nm.

Storage. Store protected from moisture.

Clomifene Citrate

Clomiphene Citrate



$C_{26}H_{28}ClNO,C_6H_8O_7$ Mol. Wt. 598.1

Clomifene citrate is a mixture of *E*- and *Z*-isomers of 2-[4-(2-chloro-1,2-diphenylvinyl)phenoxy]triethylamine dihydrogen citrate.

Clomifene Citrate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{26}H_{28}ClNO,C_6H_8O_7$, calculated on the anhydrous basis.

Category. Ovulation inducer.

Description. A white or pale yellow, crystalline powder.

Identification

Tests B and C may be omitted if test A is carried out. Test A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clomifene citrate IPRS* or with the reference spectrum of clomifene citrate.

B. Dissolve about 5 mg in 5 ml of a mixture of 10 volumes of *acetic anhydride* and 50 volumes of *pyridine* and heat in a water-bath; a deep red colour is produced.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

NOTE — In the following tests, the solutions should be protected from light in amber-coloured glassware. Ensure minimum exposure of the solutions to daylight until they are required for chromatography.

Related substances. Determine by liquid chromatography (2.4.14)

Test solution. A 0.125 per cent w/v solution of the substance under examination in the mobile phase.

Reference solution (a). A solution containing 0.125 per cent w/v of *clomifene citrate for performance test IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with butylsilane bonded to porous silica (Such as Vydac C4),
- mobile phase: mix 400 volumes of acetonitrile with 600 volumes of *water* and add 8 ml of *diethylamine*, adjust the pH of the mixture to 6.2 by the addition of about 1 to 2 ml of *phosphoric acid* taking care to reduce progressively the volume of each addition as the required pH is approached,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 233 nm,
- injection volume: 10 µl.

Equilibrate the column with the mobile phase at a flow rate of 1.2 ml per minute for about one hour.

Inject reference solution (a). Continue the chromatography for twice the retention time of the principal peak. Measure the height (A) above the baseline of the peak due to clomifene impurity A and the height (B) above the baseline of the lowest point of the curve separating this peak from the peak due to clomifene. The test is not valid unless A is greater than 15 times B and the chromatogram obtained resembles the reference chromatogram. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject reference solution (b) and the test solution. Continue the chromatography for four times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak due to 2-[4-(1,2-diphenylvinyl) phenoxy]triethylamine is not greater than that of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and the area of any other secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent); the sum of the areas of any secondary peaks is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with a retention time relative to the clomifene peak of 0.2 or less and any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

***Z*-isomer**. 30 to 50 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 25 ml of *0.1 M hydrochloric acid*, add 5 ml of *1 M sodium hydroxide* and shake with three quantities, each of 25 ml, of *ethanol-free chloroform*. Wash the combined extracts with 10 ml of *water*, dry over *anhydrous sodium sulphate* and dilute to 100.0 ml with *ethanol-free chloroform*. To 20.0 ml of the solution add 0.1 ml of *triethylamine* and dilute to 100.0 ml with *hexane*.

Reference solution. Dissolve 25 mg of *clomifene citrate IPRS* in 25 ml of *0.1 M hydrochloric acid*, add 5 ml of *1 M sodium hydroxide* and shake with three quantities, each of 25 ml, of *ethanol-free chloroform*. Wash the combined extracts with 10 ml of *water*, dry over *anhydrous sodium sulphate* and dilute to 100.0 ml with *ethanol-free chloroform*. To 20.0 ml of the solution add 0.1 ml of *triethylamine* and dilute to 100.0 ml with *hexane*.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with porous silica particles (10 µm) (Such as Parasol),
- mobile phase: a mixture of 1 volume of *triethylamine*, 200 volumes of *ethanol-free chloroform* and 800 volumes of *hexane*,

– flow rate: 2 ml per minute,
– spectrophotometer set at 302 nm,
– injection volume: 50 µl.

Equilibrate the column with the mobile phase for about 2 hours.

Inject the reference solution. The chromatogram obtained shows a peak due to *E*-isomer just before a peak due to *Z*-isomer. The test is not valid unless the resolution between the peaks corresponding to *E*- and *Z*-isomers is at least 1.0. If necessary, adjust the relative proportions of ethanol-free chloroform and hexane in the mobile phase. Measure the area of the peak due to the *Z*-isomer in the chromatogram obtained with the test solution and the reference solution.

Calculate the content of *Z*-isomer as a percentage of the total clomifene citrate present.

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.5 g and dissolve in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05981 g of $C_{26}H_{28}ClNO,C_6H_8O_7$.

Clomifene Tablets

Clomifene Citrate Tablets; Clomiphene Citrate Tablets; Clomiphene Tablets

Clomifene Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of clomifene citrate, $C_{26}H_{28}ClNO,C_6H_8O_7$.

Usual strengths. 25 mg; 50 mg.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 235 nm and 292 nm.

B. Dissolve a quantity of the powdered tablets containing 5 mg of Clomifene Citrate in 5 ml of a mixture of 10 volumes of *acetic anhydride* and 50 volumes of *pyridine* and heat in a water-bath; a deep red colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of $C_{26}H_{28}ClNO,C_6H_8O_7$ in the medium taking 317 as the specific absorbance at 232 nm.

Q. Not less than 70 per cent of the stated amount of $C_{26}H_{28}ClNO,C_6H_8O_7$.

***Z*-isomer**. 30 to 50 per cent of the content of clomifene citrate as determined in the Assay.

Determine by liquid chromatography (2.4.14)

Test solution. Shake a quantity of the powdered tablets containing about 50 mg of Clomifene Citrate with 50 ml of *0.1 M hydrochloric acid* for 10 minutes and filter. To 25 ml of the filtrate add 5 ml of *1 M sodium hydroxide* and extract with three quantities, each of 25 ml, of *ethanol-free chloroform*. Wash the combined extracts with 10 ml of *water*, dry over *anhydrous sodium sulphate* and add sufficient *ethanol-free chloroform* to produce 100.0 ml. To 20.0 ml of the resulting solution add 0.1 ml of *triethylamine* and sufficient *hexane* to produce 100 ml.

Chromatographic system
– a stainless steel column 30 cm x 4 mm, packed with porous silica particles (10 µm) (Such as Parasol),
– mobile phase: a mixture of *ethanol-free chloroform* and *hexane*, each containing 0.10 per cent v/v of *triethylamine*, adjusted so that the baseline separation is obtained between *E*- and *Z*-isomers of clomifene (a mixture of 20 volumes of *ethanol-free chloroform* and 80 volumes of *hexane* is suitable),
– flow rate: 2 ml per minute,
– spectrophotometer set at 302 nm,
– injection volume: 50 µl.

Stabilise the system by passing about 250 ml of the mobile phase.

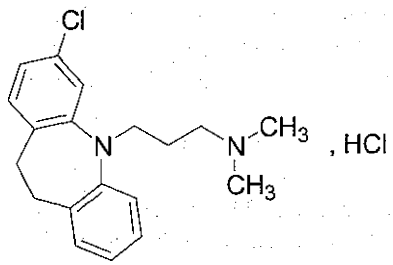
Inject the test solution. In the chromatogram a peak due to the *E*-isomer precedes that due to the *Z*-isomer of clomifene. The test is not valid unless baseline separation is achieved between *E*- and *Z*-clomifene and the column efficiency is greater than 10,000 theoretical plates per metre determine using the peak due to E-isomer.

Calculate the percentage of *Z*-isomer from the expression $100A_Z/(1.08A_E+A_Z)$ where A_Z and A_E are the areas of the peaks due to the *Z*- and *E*-isomers respectively.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 50 mg of Clomifene Citrate, shake for 30 minutes with 70 ml of *0.1 M hydrochloric acid* prepared in a 30 per cent v/v solution of *2-propanol* (instead of water normally used for the purpose as solvent), dilute to 100.0 ml with the propanolic hydrochloric acid and filter. Dissolve 5.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 292 nm (2.4.7), using a solution prepared by diluting 5 ml of the propanolic hydrochloric acid to 100 ml with *0.1 M hydrochloric acid* as the blank. Calculate the content of $C_{26}H_{28}ClNO,C_6H_8O_7$ taking 175 as the specific absorbance at 292 nm.

Clomipramine Hydrochloride



$C_{19}H_{23}ClN_2,HCl$ Mol. Wt. 351.3

Clomipramine is 3-(3-chloro-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-5-yl)-*N*,*N*-dimethylpropan-1-amine hydrochloride.

Clomipramine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{19}H_{23}ClN_2,HCl$, calculated on the dried basis.

Category. Antidepressant.

Description. A white or slightly yellow, crystalline powder, slightly hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clomipramine hydrochloride IPRS* or with the reference spectrum of clomipramine hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 5 volumes of *ammonia*, 25 volumes of *acetone* and 75 volumes of *ethyl acetate*.

Test solution. Dissolve 20 mg of the substance under examination in 10.0 ml of *methanol*.

Reference solution. A 0.2 per cent w/v solution of *clomipramine hydrochloride IPRS* in *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air, spray with 0.5 per cent solution of *potassium dichromate* in a 20 per cent solution of *sulphuric acid*. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

C. Dissolve about 5 mg in 2 ml of *nitric acid*. An intense blue colour develops.

D. Dissolve about 50 mg in 5 ml of *water* and add 1 ml of *dilute ammonia*. Allow to stand for 5 minutes and filter. Acidify the filtrate with *dilute nitric acid*. The solution gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 10 per cent w/v solution in *carbon dioxide-free water* (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution YS3 (2.4.1).

pH (2.4.24). 3.5 to 5.0, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of mobile phase B and 75 volumes of mobile phase A.

Test solution. Dissolve 20 mg of the substance under examination in 10.0 ml of solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of *clomipramine hydrochloride IPRS* in solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with cyanopropylsilyl silica (5 µm),
- mobile phase: A. 1.2 g of *sodium dihydrogen phosphate* in *water*, add 1.1 ml of *nonylamine*, adjusted to pH 3.0 with *phosphoric acid* and dilute to 1000 ml with *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
10	65	35
32	65	35
44	75	25

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 2 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, dissolve in 50.0 ml of *ethanol* and add 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*. Determine the end-point potentiometrically (2.4.25).

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03513 g of $C_{19}H_{24}Cl_2N_2$.

Storage. Store protected from light and moisture.

Clomipramine Capsules

Clomipramine Hydrochloride Capsules

Clomipramine Capsules contain Clomipramine Hydrochloride.

Clomipramine Capsules contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clomipramine hydrochloride, $C_{19}H_{23}ClN_2,HCl$.

Usual strengths. 25 mg; 75 mg; 100 mg.

Identification

Triturate a quantity of the contents of the capsules containing 0.15 g of Clomipramine Hydrochloride with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clomipramine hydrochloride IPRS* or with the reference spectrum of clomipramine hydrochloride.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate suitably diluted with the medium, if necessary, at the maximum at about 252 nm (2.4.7). Calculate the content of $C_{19}H_{23}ClN_2,HCl$ in the medium from the absorbance obtained from a solution of known concentration of *clomipramine hydrochloride IPRS* in the dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{19}H_{23}ClN_2,HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the mixed contents of 20 capsules containing 20 mg of Clomipramine Hydrochloride with 5 ml of mobile phase A with the aid of ultrasound for 15 minutes, dilute to 10.0 ml with the same solvent and filter.

Reference solution (a). A 0.2 per cent w/v solution of *clomipramine hydrochloride IPRS* in mobile phase A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with cyanopropylsilyl silica (5 µm) (Such as Hypersil BDS CN),
- mobile phase: A. a mixture of 75 volumes of solution prepared by dissolving 1.2 g of *sodium dihydrogen orthophosphate* in 950 ml of *water*, add 1.1 ml of *nonylamine*, adjusted to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 ml (solution A) and 25 volumes of *acetonitrile*,
  B. a mixture of 65 volumes of solution A and 35 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
10	65	35
32	65	35
44	75	25
50	75	25

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

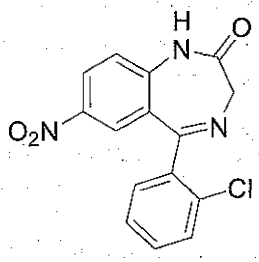
Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b)

(0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Other test. Comply with the tests stated under Capsules.

Assay. Shake a quantity of the mixed contents of 20 capsules containing 50 mg of Clomipramine Hydrochloride with 200 ml of *0.1M hydrochloric acid* for 1 hour, dilute to 250 ml with 0.1 *M hydrochloric acid* and filter. Dilute 15 ml of the filtrate to 100 ml with *0.1M hydrochloric acid.* Measure the absorbance of the resulting solution at 252 nm, (2.4.7). Calculate the content of $C_{19}H_{23}ClN_2,HCl$, taking 226 as specific absorbance at the maximum at 252 nm.

Clonazepam



$C_{15}H_{10}ClN_3O_3$ Mol. Wt. 315.7

Clonazepam is 5-(2-chlorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Clonazepam contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{15}H_{10}ClN_3O_3$, calculated on the dried basis.

Category. Anticonvulsant.

Description. A slightly yellowish, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clonazepam IPRS* or with the reference spectrum of clonazepam

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of *water*.

Test solution. Dissolve 0.05 g of the substance under examination in 10 ml of *methanol* and dilute to 100 ml with the solvent mixture.

Reference solution (a). Dissolve 25 mg of *clonazepam IPRS* in 5 ml of *methanol* and dilute to 50 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of a 0.66 per cent solution of *ammonium phosphate* previously adjusted to pH 8.0 with a 4 per cent w/v of *sodium hydroxide* or *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of *water*.

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *clonazepam IPRS* in the solvent mixture.

Use the chromatographic system as described under Related substances with injection volume 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{10}ClN_3O_3$.

Storage. Store protected from light.

Clonazepam Injection

Clonazepam Injection is a sterile solution of Clonazepam. It is prepared immediately before use by diluting Sterile

Clonazepam Concentrate with Water for Injections in accordance with the manufacturer's instructions.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The injection complies with the requirements stated under Parenteral Preparations (Injections).

Sterile Clonazepam Concentrate

Sterile Clonazepam Concentrate is a sterile solution of clonazepam in a suitable solvent.

The concentrate complies with the requirements of Concentrate for Injections stated under Parenteral Preparations and with the following requirements.

Sterile Clonazepam Concentrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clonazepam, $C_{15}H_{10}ClN_3O_3$.

Description. A clear, colourless or slightly greenish yellow solution.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2 volumes of *strong ammonia solution*, 15 volumes of *n-heptane*, 30 volumes of *nitromethane* and 60 volumes of *ether*.

Test solution. Dilute a volume of the injection containing 3 mg of Clonazepam in a stoppered tube with an equal volume of *water*, shake with 1 ml of *chloroform*, allow to separate and use the chloroform layer.

Reference solution. Dissolve 3 mg of *clonazepam IPRS* in 1 ml of *chloroform*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. After development, dry the plate in a current of cold air, spray with *2 M sodium hydroxide* and heat at 120° for 15 minutes. The principal spot in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with reference solution.

Tests

pH (2.4.24). 3.4 to 4.3.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 20 volumes of *chloroform* and 80 volumes of *ether*.

Test solution. Dilute, if necessary, a volume of the solution containing 10 mg of Clonazepam to 20 ml with *water* and extract with three 3 ml quantities of *chloroform*. Wash each *chloroform* extract separately with the same 10 ml volume of *water*, combine the extracts and add sufficient *chloroform* to produce 10 ml.

Reference solution (a). A 0.0005 per cent w/v solution of *2-amino-2'-chloro-5-nitrobenzophenone IPRS* (nitrobenzophenone) in *chloroform*.

Reference solution (b). A 0.0002 per cent w/v solution of *2-amino-2'-chloro-5-nitrobenzophenone IPRS* (nitrobenzophenone) in *chloroform*.

Reference solution (c). A 0.0002 per cent w/v of *3-amino-4-(2-chlorophenyl)-6-nitroquinolin-2-one IPRS* (carbostyril) in *chloroform*.

Apply to the plate 50 µl of each solution. After development, dry the plate in a current of cold air. For the second development use a mixture of 10 volumes of *ether* and 90 volumes of *nitromethane*. After development, dry the plate, heat at a pressure of 2 kPa at 120° for 3 hours, allow to cool and spray with a 10 per cent w/v solution of *zinc chloride* in *0.1 M hydrochloric acid*. Dry the plate in air and examine in visual light. Any spots in the chromatogram obtained with test solution corresponding to the nitrobenzophenone and carbostyril impurities are not more intense than the spots in the chromatograms obtained with reference solution (b) and (c) respectively (0.2 per cent). Any other secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Assay. *Protect the solutions from light throughout the assay.*

To a volume of injection containing 20 mg of Clonazepam, dilute to 100.0 ml with *propan-2-ol*. Dilute 10.0 ml of the solution to 100.0 ml with *propan-2-ol*. Measure the absorbance of the resulting solution at the maximum at 310 nm (2.4.7). Calculate the content of $C_{15}H_{10}ClN_3O_3$ taking 364 as the specific absorbance at 310 nm.

Storage. Store protected from light.

Labelling. The label states (1) 'Sterile Clonazepam Concentrate'; (2) that the diluted injection is to be given by intravenous injection.

Clonazepam Tablets

Clonazepam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clonazepam, $C_{15}H_{10}ClN_3O_3$.

Usual strengths. 0.5 mg; 1 mg; 2 mg.

Identification

A. To an amount of the finely powdered tablets containing about 10 mg of Clonazepam in a small separator add 25 ml of *water*, shake for 2 minutes, and extract with two quantities, each of 40 ml, of *chloroform*. Pass the extracts through *anhydrous sodium sulphate*, combine them, and evaporate to dryness at room temperature with the aid of a stream of nitrogen; the residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clonazepam IPRS* or with the reference spectrum of clonazepam.

B. In the Assay, the chromatogram obtained with the test solution corresponds to the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of degassed *water*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, discarding about 10 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. A 0.005 per cent w/v solution of *clonazepam IPRS* in *methanol*. Dilute suitably with the dissolution medium to produce a solution with a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *water*, 30 volumes of *methanol* and 30 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Q. Not less than 70 per cent of the stated amount of $C_{15}H_{10}ClN_3O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 10 mg of Clonazepam, dissolve in 75 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture and filter.

Reference solution (a). Dissolve 25 mg of *clonazepam IPRS* in 150 ml of the solvent mixture and dilute to 250.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of a 0.66 per cent w/v solution of *ammonium phosphate*, adjusted to pH 8.0 with 4 per cent w/v solution of *sodium hydroxide* or *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent). Ignore any peaks with an area 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

Test solution. To one tablet add 5 ml of the solvent mixture, disperse with the aid of ultrasound for 10 minutes, dilute, if necessary, with sufficient of the solvent mixture to produce a solution containing 0.01 per cent of Clonazepam and filter.

Calculate the content of $C_{15}H_{10}ClN_3O_3$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 10 mg of Clonazepam, dissolve in 75

ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture and filter.

Reference solution. Dissolve 25 mg of *clonazepam IPRS* in 150 ml of the solvent mixture and dilute to 250.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *tetrahydrofuran*, 42 volumes of *methanol* and 48 volumes of a 0.66 per cent w/v solution of *ammonium phosphate*, adjusted to pH 8.0 with 4 per cent w/v solution of *sodium hydroxide* or *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

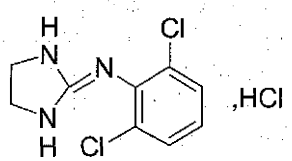
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{10}ClN_3O_3$ in the tablets.

Storage. Store protected from moisture.

Clonidine Hydrochloride



$C_9H_9Cl_2N_3,HCl$ Mol. Wt. 266.6

Clonidine Hydrochloride is 2-[(2,6-dichlorophenyl)imino]-imidazolidine hydrochloride.

Clonidine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_9Cl_2N_3,HCl$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clonidine hydrochloride IPRS* or with the reference spectrum of clonidine hydrochloride.

B. When examined in the range 230 nm to 600 nm, a 0.03 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima, at about 272 nm and 279 nm and an inflection at about 265 nm (2.4.7)

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 4.0 to 5.0, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution (b). Dissolve 5 mg of *clonidine impurity B IPRS (1-acetyl-2-[(2,6-dichlorophenyl)amino]-4,5-dihydro-1H-imidazole IPRS)* in 2 ml of *acetonitrile* and dilute to 5.0 ml with mobile phase A. To 1.0 ml of the solution, add 1.0 ml of the test solution and dilute to 10.0 ml with mobile phase A.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with propylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. dissolve 4 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 4.0 with *orthophosphoric acid*,
  B. a mixture of 25 volumes of mobile phase A and 75 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
15	30	70
15.1	90	10
20	90	10

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to clonidine and clonidine impurity B is not less than 5.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.2 g and dissolve in 70 ml of *ethanol (95 per cent)*. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.02666 g of $C_9H_9Cl_2N_3,HCl$.

Clonidine Injection

Clonidine Hydrochloride Injection

Clonidine Injection is a sterile solution of Clonidine Hydrochloride in Water for Injections.

Clonidine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clonidine hydrochloride, $C_9H_9Cl_2N_3,HCl$.

Usual strength. 150 µg in 1 ml.

Identification

A. Dilute a volume containing 300 µg of Clonidine Hydrochloride to 5 ml with *0.01 M hydrochloric acid*. When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 272 nm and 279 nm and an inflection at about 265 nm (2.4.7).

B. To a volume containing 150 µg of Clonidine Hydrochloride add 1 ml of a 10 per cent w/v solution of *ammonium reineckate* and keep aside for 5 minutes; a pink precipitate is obtained.

Tests

pH (2.4.24). 4.0 to 7.0.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. Shake together 50 volumes of *water*, 40 volumes of *1-butanol* and 10 volumes of *glacial acetic acid* and allow the layers to separate. Use the filtered upper layer.

Test solution. Add 10 ml of *methanol* to a volume containing 750 µg of Clonidine Hydrochloride, evaporate to dryness and dissolve the residue in 0.5 ml of *methanol*.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and spray with *modified potassium iodobismuthate solution*. Allow to dry in air for 1 hour, spray again with the same reagent and immediately spray with a 5 per cent w/v solution of *sodium nitrite*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume of the injection containing 150 µg of Clonidine Hydrochloride add 25 ml of *citrophosphate buffer pH 7.6*, 5 ml of *water*, and 1 ml of a solution containing 0.15 per cent w/v of *bromothymol blue* and 0.15 per cent w/v of *anhydrous sodium carbonate*. Add 30 ml of *chloroform*, shake for 1 minute and centrifuge. To 15.0 ml of the chloroform layer add 10 ml of *boric acid solution* and measure the absorbance of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a solution prepared by diluting 10 ml of *boric acid solution* to 25.0 ml with *chloroform*. Repeat the operation by adding to 5.0 ml of a 0.003 per cent w/v solution of *clonidine hydrochloride IPRS*, previously dried to constant weight at 105°, 20 ml of *citrophosphate buffer pH 7.6* and completing the procedure described above beginning at the words "5 ml of *water*". Calculate the content of $C_9H_9Cl_2N_3,HCl$ from the absorbance obtained using *clonidine hydrochloride IPRS* in place of the substance under examination.

Storage. Store in single dose containers.

Clonidine Tablets

Clonidine Hydrochloride Tablets

Clonidine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clonidine hydrochloride, $C_9H_9Cl_2N_3,HCl$.

Usual strengths. 25 µg; 100 µg; 300 µg

Identification

To a quantity of the powdered tablets containing 500 µg of Clonidine Hydrochloride add 30 ml of *water* and 5 ml of *1 M sodium hydroxide*. Swirl gently and extract with 20 ml of *chloroform*. Remove the chloroform layer, dry with *anhydrous*

sodium sulphate, filter and evaporate the filtrate to dryness. Dissolve the residue in 8 ml of *0.01 M hydrochloric acid*. The resulting solution complies with the following tests.

A. When examined in the range 230 nm to 360 nm, it shows absorption maxima at about 272 nm and 279 nm and an inflection at about 265 nm (2.4.7).

B. To 2 ml add 1 ml of a 10 per cent w/v solution of *ammonium reineckate* and allow to stand for 5 minutes; a pink precipitate is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.01 M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *clonidine hydrochloride IPRS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the test solution.

Use the chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_9Cl_2N_3$,HCl in the medium.

Q. Not less than 75 per cent of the stated amount of $C_9H_9Cl_2N_3$,HCl.

Uniformity of content. Complies with the test stated under Tablets.

For tablets containing 300 µg or more of Clonidine Hydrochloride—

To one tablet add 20 ml of *citrophosphate buffer pH 7.6*, shake until disintegrated and dilute with *citrophosphate buffer pH 7.6*, if necessary, to give a solution containing about 0.0015 per cent w/v of Clonidine Hydrochloride. To 5 ml of the supernatant liquid add 1 ml of a solution containing 0.15 per cent w/v of *bromothymol blue* and 0.15 per cent w/v of *anhydrous sodium carbonate*. Add 10 ml of *chloroform*, shake for 1 minute and centrifuge. To 5 ml of the chloroform layer add 5 ml of *boric acid solution* and measure the absorbance of a 2-cm layer of the resulting solution at the maximum at about 420 nm (2.4.7), using as the blank a mixture of 5 ml of *boric acid solution* and 5 ml of *chloroform*. Repeat the operation by using a solution prepared by diluting 5 ml of a 0.03 per cent w/v solution of *clonidine hydrochloride IPRS* to 100 ml with *citrophosphate buffer pH 7.6*, transferring 5 ml to a separator and completing the procedure described above beginning at the words "add 1 ml of a solution...".

Calculate the content of $C_9H_9Cl_2N_3$,HCl in the tablet from the absorbance obtained using *clonidine hydrochloride IPRS* in place of the tablet.

For tablets containing less than 300 µg of Clonidine Hydrochloride—

Use the same procedure but with a concentration of 0.001 per cent w/v or 0.0005 per cent w/v of Clonidine Hydrochloride as appropriate and with corresponding smaller concentrations of *clonidine hydrochloride IPRS*.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder equivalent to 0.1 mg of clonidine hydrochloride to a 100 ml volumetric flask. Add about 60 ml of mobile phase, shake by mechanical means for 15–30 min, dilute with mobile phase to volume, and mix. Centrifuge a portion of the solution to obtain a clear solution.

Reference solution (a). A 0.01 per cent w/v solution of *clonidine hydrochloride IPRS* in mobile phase.

Reference solution (b). Dilute reference solution (a) to obtain a solution of 0.0001 per cent w/v of clonidine hydrochloride in mobile phase.

Reference solution (c). A 0.0012 per cent w/v solution of *2,6-dichloroaniline* in mobile phase.

Reference solution (d). Dilute reference solution (a) and reference solution (c) to obtain 0.0002 per cent w/v solution of clonidine hydrochloride and 0.00024 per cent w/v solution of 2,6-dichloroaniline in mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with deactivated for basic compounds octylsilane bonded to porous silica(5 µm),
- mobile phase: a mixture of 500 volumes of *methanol*, 500 volumes of 0.22 per cent w/v solution of *sodium octanesulphonate* in *water* and 1 volume of *phosphoric acid*, adjusted to pH 3.0 with *1 M sodium hydroxide*.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

Inject reference solution (b). The test is not valid unless the relative standard deviation of clonidine peak is not more than 2.0 per cent.

Inject reference solution (d). The relative retention time for clonidine and 2,6-dichlroaniline are about 0.5 and 1.0 respectively. The test is not valid unless the theoretical plate is not less than 3500 and the tailing factor of clonidine peak is not more than 1.5.

Inject reference solution (b) and the test solution.

Calculate the content of $C_9H_9Cl_2N_3,HCl$ in the tablets.

Clonidine Hydrochloride and Chlorthalidone Tablets

Clonidine Hydrochloride and Chlorthalidone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of clonidine hydrochloride, $C_9H_9Cl_2N_3,HCl$ and chlorthalidone, $C_{14}H_{11}ClN_2O_4S$.

Usual strengths. Clonidine Hydrochloride, 0.1 mg and Chlorthalidone, 15 mg; Clonidine Hydrochloride, 0.2 mg and Chlorthalidone, 15 mg.

Identification

A. Weigh a quantity of powdered tablets containing about 1 mg of clonidine hydrochloride to a beaker. Add 30 ml of *water*, stir for 5 minutes and pass through a filter of medium pore size into a sintered-glass funnel. Transfer the filtrate in to a separator, add 2.0 ml of *0.1 M sodium hydroxide* and extract with 10.0 ml of *chloroform*, collect the chloroform extract in to separator. Extract the chloroform layer with 5.0 ml of *0.01M hydrochloric acid*, collect the acid extract in a beaker. Remove any residual chloroform from the acid extract by heating on a water-bath.

When examined in the range 200 nm to 400 nm (2.4.7), the test solution exhibits maximum and minimum adsorption at the same wavelengths with that obtained with *clonidine hydrochloride IPRS* treated in the similar manner.

B. Powder 10 tablets and transfer into a 50 ml beaker. Add 10 ml of *methanol*, boil on a water-bath for 5 minutes, and filter. Add 20 ml of *water* to the filtrate, and boil on a water-bath for 5 minutes. Cool with stirring in ice until crystals forms. Filter the crystals and dry at 105° for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chlorthalidone IPRS* or with the reference spectrum of chlorthalidone.

C. In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Take 20 ml of the filtrate into a 25.0 ml volumetric flask and dilute with 0.5 per cent w/v solution of *monobasic ammonium phosphate* to volume.

Reference solution (a). A 0.001 per cent w/v solution of *clonidine hydrochloride IPRS* in 0.5 per cent w/v solution of *monobasic ammonium phosphate.*

Reference solution (b). A 0.015 per cent w/v solution of *chlorthalidone IPRS* in *methanol.*

Reference solution (c). Dilute reference solution (a) and (b) with 0.5 per cent w/v solution of *monobasic ammonium phosphate* to obtain a solution having a known concentration similar to the test solution.

Use chromatographic system as described under Assay.

Calculate the content of $C_{14}H_{11}ClN_2O_4S$ and $C_9H_9Cl_2N_3,HCl$.

Q. Not less than 50 per cent of the stated amount of $C_{14}H_{11}ClN_2O_4S$ and not less than 80 per cent of the stated amount of $C_9H_9Cl_2N_3,HCl$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions and the reference solution described under Assay.

Test solution. Transfer one tablet to a 100.0 ml volumetric flask. Add 10.0 ml of *methanol*, disperse with the aid of ultrasound for 5 minutes, add 50.0 ml buffer solution and sonicate for 5 minutes. Allow to cool and diluted to volume with buffer solution. Mix well and centrifuge.

Reference solution (d). Dilute 1.0 ml of reference solution (a) to 100.0 ml with buffer solution.

Inject reference solution (d) and the test solution.

Calculate the content of $C_9H_9Cl_2N_3,HCl$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. A 0.1 per cent w/v solution of *monobasic ammonium phosphate* in *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 15 mg of chlorthalidone to a 100-ml volumetric flask, add 10.0 ml of *methanol* and sonicate for 5

minutes. Add 40.0 ml of buffer solution and sonicate until the solution is free from agglomerates. Allow to cool and dilute to volume. Mix and centrifuge.

Reference solution (a). A 0.01 per cent w/v solution of *clonidine hydrochloride IPRS* in *methanol*.

Reference solution (b). A 0.15 per cent w/v solution of *chlorthalidone IPRS* in *methanol*.

Reference solution (c). Dilute reference solution (a) and (b) with buffer solution to obtain a solution having a known concentration similar to the test solution.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm packed with octaylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 100 volumes of *methanol*, 100 volumes of *acetonitrile* and 800 volumes of buffer solution,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

The relative retention times with respect to chlorthalidone for clonidine hydrochloride is about 0.2.

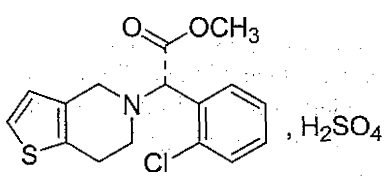
Inject reference solution (c). The test is not valid unless the resolution between the peaks due to clonidine hydrochloride and chlorthalidone is not less than 3.0 and the relative standard deviation for replicate injections for each of the peaks corresponding to clonidine hydrochloride and chlorthalidone is not more than 2.0 per cent.

Inject reference solution (c) and the test solution.

Calculate the contents of $C_{14}H_{11}ClN_2O_4S$ and $C_9H_9Cl_2N_3,HCl$ in the tablets.

Storage. Store protected from moisture.

Clopidogrel Bisulphate



$C_{16}H_{16}ClNO_2S, H_2SO_4$ Mol. Wt. 419.9

Clopidogrel Bisulphate is methyl (*S*)-α-(*o*-chlorophenyl)-6,7-dihydrothieno[3,2-*c*]pyridine-5-(4*H*)-acetate sulphate.

Clopidogrel Bisulphate contains not less than 97.0 per cent and not more than 101.5 per cent of $C_{16}H_{16}ClNO_2S,H_2SO_4$, calculated on the dried basis.

Category. Antithrombotic.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clopidogrel bisulphate IPRS* or with the reference spectrum of clopidogrel bisulphate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

C. Gives reaction A of sulphates (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — For all clopidogrel related compounds, the concentrations are expressed as bi-sulphate salts. Use bi-sulphate salt equivalents stated on reference substances labels to calculate the concentrations as appropriate.

Test solution. Dissolve 100 mg of the substance under examination in 5.0 ml of *methanol* and dilute to 200.0 ml with the mobile phase.

Reference solution. A solution containing 20 µg per ml of *clopidogrel bisulphate IPRS*, 40 µg per ml of *(+)-(S)-(o-chloro-phenyl)-6,7-dihydrothieno(3,2-c]pyridine-5(4H)-acetic acid IPRS* (*clopidogrel impurity A IPRS)*, 120 µg per ml of *methyl(±)-(o)-chlorophenyl)-4,5 dihydrothieno (2,3-c)pyridine-6(7H)-acetate, hydrochloride IPRS (clopidogrel impurity B IPRS)*, and 200 µg per ml of *methyl (-)-(R)-o-chlorophenyl)-6,7 dihydrothieno(3,2-c)pyridine-5(4H)-acetate,hydrogen sulphate IPRS (clopidogrel impurity C IPRS)*, in *methanol*. Dilute 5.0 ml of the solution to 200.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with chiral recognition protein, ovomucoid, chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium phosphate* in 1000 ml of *water* and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The relative retention time with respect to clopidogrel, for impurity A is about 0.5, for enantiomers of clopidogrel impurity B, about 0.8 and 1.2 and for clopidogrel impurity C, about 2.0.

Inject the reference solution. The test is not valid unless the resolution between clopidogrel and the first enantiomer of clopidogrel impurity B is not less than 2.5.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of the peak due to clopidogrel impurity A is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of the peak due to the first enantiomer of clopidogrel impurity B, multiplied with correction factor 0.5 is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.3 per cent) and the area of the peak due to clopidogrel impurity C is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (1.0 per cent), the area of any peak due to other impurities is not more than the area of the peak due to *clopidogrel bisulphate IPRS* in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of all the impurities is not more than 1.5 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 100 mg of the substance under examination and dissolve in 100.0 ml of *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *clopidogrel bisulphate IPRS* in *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). A solution containing 100 µg per ml of *clopidogrel bisulphate IPRS* and 200 µg per ml of *clopidogrel impurity B IPRS* in *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with chiral recognition protein, ovomucoid, chemically bonded to porous silica (5 µm),
- mobile phase : a mixture of 75 volumes of a buffer solution prepared by dissolving 1.36 g of *monobasic potassium phosphate* in 1000 ml of *water* and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Inject reference solution (b). The relative retention times with respect to clopidogrel, for enantiomers of clopidogrel impurity B are about 0.8 and 1.2 and the resolution between clopidogrel and the first enantiomer of clopidogrel impurity B is not less than 2.5.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{16}ClNO_2S,H_2SO_4$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Clopidogrel Tablets

Clopidogrel Bisulphate Tablets

Clopidogrel Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clopidogrel, $C_{16}H_{16}ClNO_2S$.

Usual strength: 75 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{16}H_{16}ClNO_2S$ in the medium from the absorbance obtained from a solution of known concentration of clopidogrel prepared by dissolving a suitable quantity of *clopidogrel bisulphate IPRS* in 20 ml of *methanol* and further diluting with the medium.

Q. Not less than 80 per cent of the stated amount of $C_{16}H_{16}ClNO_2S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 75 mg of Clopidogrel, add 5 ml of *methanol* and dilute to 200.0 ml with the mobile phase. Allow to stand for 10 minutes and mix and filter.

Reference solution (a). A solution containing *clopidogrel bisulphate IPRS* equivalent to 40 µg per ml of clopidogrel, 250 µg per ml of *[(+)-(S)-(o-chlorophenyl)-6,7-dihydro-thieno(3,2-c]pyridine-5(4H)-acetic acid] IPRS (clopidogrel impurity A IPRS)* and 300 µg per ml of *[methyl(-)-(R)-o-chlorophenyl)-6,7 dihydrothieno(3,2-c)pyridine-5(4H)-acetate,hydrogen sulphate]IPRS (clopidogrel impurity C IPRS)* in *methanol*. Dilute 5.0 ml of the solution to 200.0 ml with the mobile phase.

Reference solution (b). A solution containing *clopidogrel bisulphate IPRS* equivalent to 100 µg per ml of clopidogrel, and 200 µg per ml of *[methyl(±)-(o)-chlorophenyl)-4,5 dihydro-thieno(2,3-c)pyridine-6(7H)-acetate, hydrogen sulphate] IPRS (clopidogrel impurity B IPRS)* in *methanol*. Dilute 5.0 ml of the solution to 200.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with chiral recognition protein, ovomucoid, chemically bonded to porous silica (5 µm),
- mobile phase : a mixture of 75 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium phosphate* in 1000 ml of *water* and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The relative retention times with respect to clopidogrel for enantiomers of clopidogrel impurity B are about 0.8 and 1.2, for clopidogrel impurity A is about 0.5 and for clopidogrel impurity C is about 2.0.

Inject reference solution (b). The resolution between clopidogrel and the first enantiomer of clopidogrel impurity B is not less than 2.5. Inject reference solution (a). The relative standard deviation for replicate injections is not more than 15.0 per cent for each peak.

In the chromatogram obtained with the test solution the area of the peak due to clopidogrel impurity A is not more than 0.7 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.2 per cent), and the area of the peak due to clopidogrel impurity C is not more than 0.75 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.5 per cent), the area of any peak due to other impurities is not more than 0.75 times the area of the peak due to *clopidogrel bisulphate IPRS* in the chromatogram obtained with reference solution (a) (0.2 per cent, excluding impurity B) and the sum of all the impurities is not more than 2.5 per cent, excluding impurity B.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder equivalent to 75 mg of clopidogrel, disperse in 100.0 ml of the *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the *methanol* and filter.

Reference solution (a). A 0.01 per cent w/v solution of *clopidogrel bisulphate IPRS* in *methanol*.

Reference solution (b). A solution containing *clopidogrel bisulphate IPRS* equivalent to 100 µg per ml of clopidogrel and 200 µg per ml of *[methyl(±)-(o)-chlorophenyl)-4,5 dihydro-thieno(2,3-c)pyridine-6(7H)-acetate, hydrogen sulphate] IPRS (clopidogrel impurity B IPRS)* in *methanol*. Dilute 5.0 ml of the solution to 200.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with chiral recognition protein, ovomucoid, chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 1.36 g of *monobasic potassium phosphate* in 1000 ml of *water* and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The relative retention times with respect to clopidogrel, for enantiomers of clopidogrel impurity B are about 0.8 and 1.2

Inject reference solution (b). The test is not valid unless the resolution between clopidogrel and the first enantiomer of clopidogrel impurity B is not less than 2.5.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{16}ClNO_2S$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of clopidogrel.

Clopidogrel and Aspirin Tablets

Clopidogrel and Aspirin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clopidogrel, $C_{16}H_{16}ClNO_2S$ and aspirin, $C_9H_8O_4$.

Usual strengths. Clopidogrel 300 mg and Aspirin 150 mg; Clopidogrel 75 mg and Aspirin 75 mg; Clopidogrel 75 mg and Aspirin 150 mg

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

For Clopidogrel —

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *clopidogrel bisulphate IPRS* in dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of test solution.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS, C8),
- mobile phase: a mixture of 3 volumes of *0.05 M sodium dihydrogen orthophosphate* and 1 volume of *acetonitrile*, adjusted the pH 2.0 with dilute *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

The elution order of chromatogram is aspirin and clopidogrel respectively.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution

Calculate the content of $C_{16}H_{16}ClNO_2S$ in the tablet.

1 mg of $C_{16}H_{18}ClNO_6S_2$ is equivalent to 0.7664 mg of $C_{16}H_{16}ClNO_2S$

Q. Not less than 70 per cent of the stated amount of $C_{16}H_{16}ClNO_2S$

For Aspirin —

Apparatus No. 2 (Paddle),

Medium. 900 ml of a buffer solution prepared by dissolving 2.99 g of *sodium acetate trihydrate* containing 1.7 ml of *glacial acetic acid* in 1000 ml of *water*, adjusted to pH 4.5 with *sodium hydroxide solution*.

Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared reference solution and test solution.

Test solution. Dilute the filtrate if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *aspirin IPRS* in dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of test solution.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C8),
- mobile phase: a mixture of 3 volumes of *0.05 M sodium dihydrogen orthophosphate* and 1 volume of *acetonitrile*, adjusted the pH 2.0 with *dilute orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

The elution order is Aspirin and Clopidogrel.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_8O_4$ in the tablet.

Q. Not less than 70 per cent of the stated amount of $C_9H_8O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

For Clopidogrel —

Test solution. Disperse a quantity of the powdered tablet containing about 50 mg of clopidogrel in 30 ml of mobile phase, with the aid of ultrasound for 15 minutes. Cool and dilute to 50.0 ml with the mobile phase and filter.

Reference solution (a). A 0.065 per cent w/v solution of *clopidogrel bisulphate IPRS* in the mobile phase.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 200.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C8),
- mobile phase: a mixture of 45 volumes of a buffer solution prepared by dissolving 0.33 g of *diammonium hydrogen orthophosphate* and 0.2 g of *tetrabutylammonium hydrogen sulphate* in 100 ml of *water*, adjusted the pH 7.0 and 55 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

For Aspirin —

Free salicylic acid. Not more than 3.0 per cent.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 99 volumes of *acetonitrile* and 1 volume of *formic acid*.

Test solution: Disperse a quantity of the powdered tablet containing about 300 mg of aspirin in 70 ml of the solvent mixture, with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the mobile phase and filter.

Reference solution (a). A 0.01 per cent w/v solution of *salicylic acid IPRS* in the solvent mixture.

Reference solution (b). A 0.3 per cent w/v solution of *aspirin IPRS* and *clopidogrel bisulphate IPRS* in reference solution (a).

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil C18),
- sample temperature: 5°,
- mobile phase: a mixture of 75 volumes of *0.05M* of *sodium dihydrogen orthophosphate dihydrate* and 25 volumes of *acetonitrile*, adjusted the pH 2.0 with *dilute orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

The elution order of peaks in the chromatogram is aspirin, salicylic acid and clopidogrel bisulphate respectively.

Inject reference solution (a) and (b). The test is not valid unless the resolution between salicylic acid and aspirin is not less than 2.0 and salicylic acid and clopidogrel is not less than 1.5 with reference solution (b). The column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of free salicylic acid.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 100 mg of Clopidogrel in 250-ml volumetric flask, add sufficient amount of *methanol* disperse with the aid of ultrasound for 15 minutes. Cool and dilute to volume with *methanol*, mix and filter. Dilute 5.0 ml of the filtrate to 25.0 ml with the mobile phase.

Reference solution (a). A 0.08 per cent w/v solution of *clopidogrel bisulphate IPRS* in *methanol*.

Reference solution (b). A 0.08 per cent w/v solution of *aspirin IPRS* in *methanol*.

Reference solution (c). Dilute reference solution (a) and (b) with the mobile phase to obtain a solution having similar concentration to the test solution.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C8),
- mobile phase: a mixture of 3 volumes of *0.05 M sodium dihydrogen orthophosphate* and 1 volume of *acetonitrile*, adjusted the pH 2.0 with *dilute orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

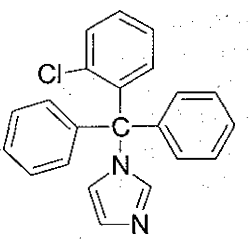
Inject reference solution (c) and the test solution.

Calculate the contents of $C_{16}H_{16}ClNO_2S$ and $C_9H_8O_4$.

1 mg of $C_{16}H_{18}ClNO_6S_2$ is equivalent to 0.7664 mg of $C_{16}H_{16}ClNO_2S$.

Storage. Store protected from moisture, at a temperature not exceeding 25°.

Clotrimazole



$C_{22}H_{17}ClN_2$ Mol. Wt. 344.8

Clotrimazole is 1-[(2-chlorophenyl)diphenylmethyl]-1*H*-imidazole.

Clotrimazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{17}ClN_2$, calculated on the dried basis.

Category. Antifungal.

Description. A white to pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clotrimazole IPRS* or with the reference spectrum of clotrimazole.

B. When examined in the range 230 nm to 360 nm, a 0.04 per cent w/v solution in a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *methanol* shows absorption maxima at about 262 and 265 nm; absorbance at about 262 nm, about 0.9 and at about 265 nm, about 0.92 (2.4.7).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *acetonitrile* and dilute to 50.0 ml with *acetonitrile*.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with *acetonitrile*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *acetonitrile*.

Reference solution (c). A solution containing 0.0002 per cent w/v, each of, *clotrimazole impurity D IPRS* and *clotrimazole impurity E IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. dissolve 1.0 g of *potassium dihydrogen phosphate* and 0.5 g of *tetrabutyl ammonium hydrogen sulphate* in *water* and dilute to 1000 ml with *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
3	75	25
25	20	80
30	20	80
35	75	25

Name	Relative retention time
Clotrimazole impurity D[1]	0.1
Clotrimazole impurity F[2]	0.9
Clotrimazole (Retention time: about 12 minutes)	1.0
Clotrimazole impurity B[3]	1.1
Clotrimazole impurity E[4]	1.5
Clotrimazole impurity A[5]	1.8

[1]imidazole,

[2]deschloroclotrimazole,

[3]1-[(4-chlorophenyl)diphenylmethyl]-1H-imidazole,

[4]2-chlorobenzophenone,

[5](2-chlorophenyl)diphenylmethanol.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and tailing factor is not more than 2.0.

Inject reference solution (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to clotrimazole impurities A and B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of peak corresponding to clotrimazole impurities D and E is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent), the area of peak corresponding to clotrimazole impurity F is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.3 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator to a green end-point. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03448 g of $C_{22}H_{17}ClN_2$.

Storage. Store protected from light.

Clotrimazole Cream

Clotrimazole Cream contains Clotrimazole in a suitable cream base.

Clotrimazole Cream contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clotrimazole, $C_{22}H_{17}ClN_2$.

Usual strengths. 1 per cent w/w; 2 per cent w/w.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. *Di-isopropyl ether* in a chromatography tank, containing 25 ml of *strong ammonia solution* in a beaker.

Test solution. Shake a quantity of the cream containing 20 mg of Clotrimazole with 4 ml of *dichloromethane* for 30 minutes, centrifuge and use the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of *clotrimazole IPRS* in *dichloromethane*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and spray with *dilute potassium iodobismuthate solution*. The spot in the chromatogram obtained with the test solution is reddish brown and corresponds to the spot in the chromatogram obtained with the reference solution.

Tests

2-Chlorotritanol. Determine by liquid chromatography (2.4.14).

Test solution. Extract a quantity of the cream containing 20 mg of Clotrimazole by warming with 20 ml of *methanol* in a water-bath at 50° for 5 minutes, shaking occasionally. Remove from the water-bath, shake the mixture vigorously while cooling to room temperature, cool in ice for 15 minutes, centrifuge for 5 minutes and decant the supernatant liquid. Repeat the extractions with two further quantities, each of 20 ml, of *methanol*. To the combined methanol extracts add 10 ml of *methanol* and dilute to 100.0 ml with *methanol*. Cool in ice and filter through a membrane filter.

Reference solution (a). A 0.0002 per cent w/v solution of *2-chlorotritanol IPRS* in *methanol*.

Reference solution (b). Dilute 1 volume of the test solution to 50 volumes with *methanol*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M phosphoric acid* adjusted to pH 7.5 with a 10 per cent w/v solution of *triethylamine* in *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 1800 theoretical plates.

Inject reference solution (a) and the test solution. Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any peak corresponding to 2-chlorotritanol in the chromatogram obtained with the test solution is not greater then the area of the peak in the chromatogram obtained with reference solution (a).

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Extract a quantity of the cream containing 25 mg of Clotrimazole by warming with 25 ml of *methanol* in a water-bath at 50° for 5 minutes, shaking occasionally. Remove from the water-bath, shake the mixture vigorously while cooling to room temperature, cool in ice for 15 minutes, centrifuge for 5 minutes and decant the supernatant liquid. Repeat the extraction with 20 ml, of *methanol*. Dilute the combined methanol extracts to 50.0 ml with *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *clotrimazole IPRS* in *methanol*.

Reference solution (b). A solution containing 0.01 per cent w/v solution, each of, *clotrimazole IPRS* and *2-chlorotritanol IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of *acetonitrile* and 25 volumes of a buffer solution prepared by dissolving 4.35 g of *dibasic potassium phosphate* in 1000 ml of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 25 µl.

The relative retention time with reference to clotrimazole for 2-chlorotritanol is about 1.2.

Inject reference solution (b). The test is not valid unless the resolution between clotrimazole and 2-chlorotritanol peaks is not less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{17}ClN_2$ in the cream.

Storage. Store at a temperature not exceeding 30°.

Clotrimazole Lotion

Clotrimazole Lotion contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clotrimazole, $C_{22}H_{17}ClN_2$.

Usual strength. 1.0 per cent w/v.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (c).

Tests

pH (2.4.24). 5.0 to 7.0.

Clotrimazole related compound A. Not more than 5.0 per cent.

Determine by liquid chromatography (2.4.14), as described under Assay.

Calculate the percentage of clotrimazole related compound A using the chromatograms of reference solution (c) and the test solution.

Microbial contamination (2.2.9). 1.0 g is free from *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Other tests. Comply with the tests stated under Lotions.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. A 0.007 per cent w/v solution of *testosterone propionate* in *dehydrated alcohol.*

Test solution. Transfer the equivalent of 10 mg of Clotrimazole from freshly mixed lotion to a screw-capped, 50-ml centrifuge tube. Add 10.0 ml of *internal standard solution*, place the cap on the tube, and heat at 50° in a water bath for 5 minutes, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 minutes. Cool in a methanol–ice bath for 15 minutes, and promptly centrifuge. Transfer the supernatant liquid to a test tube. Add 10.0 ml of *dehydrated alcohol* to the residue in the centrifuge tube, and repeat the extraction as directed above, beginning with "place the cap on the tube". Transfer the supernatant liquid to the test tube containing the supernatant liquid from the first extraction.

Reference solution (a). A 0.2 per cent w/v solution of *clotrimazole IPRS* in *dehydrated alcohol.*

Reference solution (b). A 0.01 per cent w/v solution of *clotrimazole related compound A IPRS [(o-Chlorophenyl) diphenylmethanol IPRS]* in *dehydrated alcohol.*

Reference solution (c). Mix 5.0 ml, each of, reference solution (a), reference solution (b) and 10.0 ml of the internal standard solution.

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) and a guard column 6 cm × 2.1 mm, packed with the same column material (10 µm),
- mobile phase: a mixture of 1 volume of a buffer solution prepared by dissolving 4.35 g of *dibasic potassium phosphate* in 1000 ml of *water* and 3 volumes of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The relative retention time with respect to clotrimazole for clotrimazole related compound A and testosterone propionate is about 0.9 and 1.5, respectively.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to clotrimazole related compound A and clotrimazole is not less than 1.2 and the resolution between the peaks due to clotrimazole and testosterone propionate is not less than 1.9. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{22}H_{17}ClN_2$ in the lotion.

Storage. Store protected from moisture, at a temperature between 2° to 30°.

Clotrimazole Pessaries

Clotrimazole Vaginal Tablets

Clotrimazole Pessaries contain Clotrimazole in a suitable base.

Clotrimazole Pessaries contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of clotrimazole, $C_{22}H_{17}ClN_2$.

Usual strengths. 100 mg; 200 mg; 500 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. Di-isopropyl ether in a chromatography tank, containing 25 ml of *strong ammonia solution* in a beaker.

Test solution. Shake a quantity of the powdered pessaries containing 20 mg of Clotrimazole with 4 ml of *dichloromethane* for 30 minutes, centrifuge and use the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of *clotrimazole IPRS* in *dichloromethane*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air and spray with *dilute potassium iodobismuthate solution*. The spot in the chromatogram obtained with the test solution is reddish brown and corresponds to the spot in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Add 50 ml of *methanol* to a quantity of the powdered pessaries containing 0.1 g of Clotrimazole and shake for 20 minutes. Dilute to 100 ml with *methanol* and filter. To 20 ml of the filtrate add 50 ml *methanol* and dilute to 100.0 ml with *methanol*.

Reference solution (a). A 0.0002 per cent w/v solution of *2-chlorotritanol IPRS* in *methanol*.

Reference solution (b). Dilute 1 volume of the test solution to 50 volumes with *methanol*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M orthophosphoric acid* adjusted to pH 7.5 with a 10 per cent w/v solution of *triethylamine* in *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject reference solution (b). The column efficiency, determined using the principal peak in the chromatogram obtained is not less than 1800 theoretical plates.

Inject reference solution (a) and the test solution. Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any peak corresponding to 2-chlorotritanol in the chromatogram obtained with the test solution is not greater then the area of the peak in the chromatogram obtained with reference solution (a).

Other tests. Comply with the tests stated under Pessaries.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 pessaries. Disperse a quantity of the powder containing about 0.1 g of Clotrimazole, add 50 ml of *methanol* and shake for 20 minutes, dilute to 250.0 ml with *methanol* and filter. To 10.0 ml of the filtrate add 60 ml of *methanol* and dilute to 100.0 ml with *methanol*.

Reference solution. Dissolve 20 mg of *clotrimazole IPRS* in 70 ml of *methanol*, add sufficient *0.02 M orthophosphoric acid* to produce 100.0 ml and dilute 1.0 ml of the resulting solution to 5.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of *0.02 M orthophosphoric acid*, adjusted to pH 7.5 with a 10 per cent w/v solution of *triethylamine* in *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

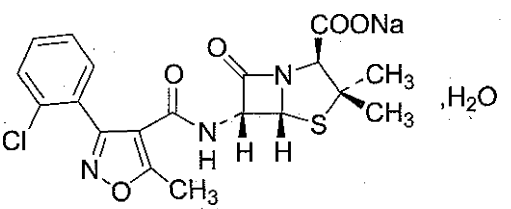
Inject the reference solution. The column efficiency, determined using the peak in the chromatogram obtained should be not less than 1800 theoretical plates.

Inject the reference solution and the test solution. Allow the chromatography to proceed for 1.5 times the retention time of the principal peak in the chromatogram obtained with the test solution.

Calculate the content of $C_{22}H_{17}ClN_2$ in the pessaries.

Storage. Store protected from moisture and crushing.

Cloxacillin Sodium



$C_{19}H_{17}ClN_3NaO_5S, H_2O$ Mol. Wt. 475.9

Cloxacillin Sodium is sodium (6*R*)-6-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]penicillanate monohydrate.

Cloxacillin Sodium contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{19}H_{17}ClN_3NaO_5S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white, crystalline powder; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin sodium IPRS* or with the reference spectrum of cloxacillin sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1); absorbance of the solution at about 430 nm, not more than 0.04 (2.4.7).

pH (2.4.24). 5.0 to 7.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +160° to +169°, determined at 20° in a 1.0 per cent w/v solution.

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v, each of, *flucloxacillin sodium IPRS* and *cloxacillin sodium IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 5.0 with *dilute sodium hydroxide solution*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cloxacillin (first peak) and flucloxacillin (second peak) is not less than 2.5.

Inject reference solution (a) and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (2.3.43). Not more than 4.5 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Prepare a *0.02 M monobasic potassium phosphate* solution and adjusted to pH 6.6 with *2 M sodium hydroxide*.

Test solution. Weigh accurately about 55 mg of the substance under examination and dilute to 100.0 ml with the buffer solution. Dilute 1.0 ml of the solution to 10.0 ml with the buffer solution.

Reference solution. Weigh a suitable quantity of *cloxacillin sodium IPRS* dissolve in the buffer solution and dilute with the buffer solution to obtain a solution containing a known concentration of about 0.0055 per cent w/v of Cloxacillin Sodium.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{17}ClN_3NaO_5S$.

Cloxacillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.40 Endotoxin Unit per mg of cloxacillin.

Cloxacillin Sodium intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of

parenteral preparations, the containers should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Cloxacillin Capsules

Cloxacillin Sodium Capsules

Cloxacillin Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

Usual strengths. The equivalent of 250 mg and 500 mg of cloxacillin.

Identification

A. Determine on the contents of the capsules by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin sodium IPRS* or with the reference spectrum of cloxacillin sodium.

B. The contents of the capsules give reactions (A) and (B) of sodium salts (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Use the filtrate, dilute if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Similarly measure the absorbance of a standard solution of known concentration of *cloxacillin sodium IPRS* in place of the contents of the capsules. Calculate the content of $C_{19}H_{18}ClN_3O_5S$.

Q. Not less than 75 per cent of the stated amount of $C_{19}H_{18}ClN_3O_5S$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Prepare a *0.02 M monobasic potassium phosphate* solution and adjusted to pH 6.6 with *2 M sodium hydroxide*.

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Cloxacillin, dissolve in the buffer solution by stirring for 10 minutes, dilute to 100.0 ml with the buffer solution. Dilute 1.0 ml of the solution to 10.0 ml with the buffer solution and filter.

Reference solution. Weigh accurately a suitable quantity of *cloxacillin sodium IPRS* dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.0055 per cent w/v of cloxacillin sodium.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{18}ClN_3O_5S$ in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of cloxacillin.

Cloxacillin Injection

Cloxacillin Sodium Injection

Cloxacillin Injection is a sterile material consisting of Cloxacillin Sodium with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cloxacillin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

Usual strengths. The equivalent of 250 mg and 500 mg of cloxacillin.

Description. A white or almost white powder; very hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin sodium IPRS* or with the reference spectrum of cloxacillin sodium.

B. It gives reaction (A) of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.0, determined in a 10 per cent w/v solution.

Bacterial endotoxins (2.2.3). Not more than 0.40 Endotoxin Unit per mg of cloxacillin.

Water (2.3.43). Not more than 4.5 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Prepare a *0.02 M monobasic potassium phosphate* solution and adjusted to pH 6.6 with *2 M sodium hydroxide.*

Test solution. Determine the weight of the content of 10 containers. Weigh accurately a suitable quantity of the mixed contents of the 10 containers containing about 50 mg of Cloxacillin, dissolve in the buffer solution by shaking and dilute to 100.0 ml with the buffer solution.

Reference solution. Weigh accurately a suitable quantity of *cloxacillin sodium IPRS* dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{18}ClN_3O_5S$ in the injection.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the quantity of Cloxacillin Sodium contained in the sealed container in terms of the equivalent amount of cloxacillin.

Cloxacillin Syrup

Cloxacillin Sodium Syrup; Cloxacillin Elixir; Cloxacillin Sodium Elixir; Cloxacillin Oral Solution; Cloxacillin Sodium Oral Solution

Cloxacillin Syrup is a mixture consisting of Cloxacillin Sodium with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Cloxacillin Syrup contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of $C_{19}H_{18}ClN_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

Usual strength. The equivalent of 125 mg of cloxacillin per 5 ml when reconstituted with water.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

The constituted suspension complies with the tests stated under Oral liquids and with the following tests.

Tests

pH (2.4.24). 4.0 to 7.0.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Prepare a *0.02 M monobasic potassium phosphate* solution and adjusted to pH 6.6 with *1 M sodium hydroxide.*

Test solution. Transfer an accurately weighed quantity of the oral suspension containing about 0.125 g of the cloxacillin to a 250-ml volumetric flask and dissolve in the buffer solution by stirring for 15 minutes, dilute to 250.0 ml with the buffer solution.

Reference solution. Weigh accurately a suitable quantity of *cloxacillin sodium IPRS* dissolve in the buffer solution and dilute to obtain a solution containing a known concentration of about 0.55 mg per ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 80 volumes of the buffer solution and 20 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

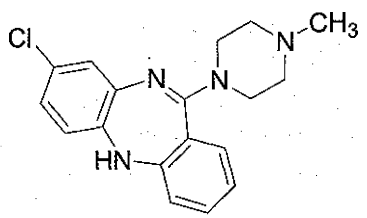
Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{19}H_{18}ClN_3O_5S$ weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of cloxacillin.

Clozapine



$C_{18}H_{19}ClN_4$ Mol. Wt. 326.8

Clozapine is 8-chloro-11-(4-methylpiperazin-1-yl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine.

Clozapine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{19}ClN_4$, calculated on the dried basis.

Category. Antipsychotic.

Description. A yellow crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *clozapine IPRS* or with the reference spectrum of clozapine.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 20 volumes of *water* and 80 volumes of *methanol*.

Test solution. Dissolve 75 mg of the substance under examination in 80 ml of *methanol* and dilute to 100.0 ml with *water*.

Reference solution. Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile*, 10 volumes of *methanol* and 80 volumes of buffer solution prepared by dissolving 2.04 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 2.4 with *orthophosphoric acid*,
  B. a mixture of 40 volumes of *acetonitrile*, 40 volumes of *methanol* and 20 volumes of buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 257 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
4	100	0
24	0	100
29	0	100
30	100	0
35	100	0

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and tailing factor is not more than 2.0 per cent. The relative retention time with reference to clozapine for 8-chloro-11-(piperazin-1-yl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine (clozapine impurity C) is about 0.9, for 1-[2-[(2-amino-4-chlorophenyl)amino]benzoyl]-4-methylpiperazine (clozapine impurity D) is about 1.1, for 8-chloro-5,10-dihydro-11*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one (clozapine impurity A) is about 1.6, for 11,11'-(piperazine-1,4-diyl)bis(8-chloro-5*H*-dibenzo[*b*,*e*][1,4]diazepine) (clozapine impurity B) is about 1.7.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the

peak due to clozapine impurity A is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent). The area of any peak due to clozapine impurity B, clozapine impurity D multiplied with correction factor 2.7, is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of the peak due to clozapine impurity C is not more than three times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 6 times the area of the principal peak in the chromatogram obtained with the reference solution (0.6 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh accurately about 0.115 g, dissolve in 70 ml of *glacial acetic acid* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01634 g of $C_{18}H_{19}ClN_4$.

Storage. Store protected from light.

Clozapine Tablets

Clozapine Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of clozapine, $C_{18}H_{19}ClN_4$.

Usual strengths. 25 mg; 50 mg; 100 mg.

Identification

A. In the Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to the principal spot obtained with the reference solution (a).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of acetate buffer pH 4.0, prepared by dissolving 2 g of *sodium hydroxide* in 450 ml of *water*, adjusted to pH 4.0 with *glacial acetic acid* and diluting with *water* to 1000 ml,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate if necessary, with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Similarly measure the absorbance of a standard solution of known concentration of *clozapine IPRS* and calculate the content of $C_{18}H_{19}ClN_4$.

Q. Not less than 80 per cent of the stated amount of $C_{18}H_{19}ClN_4$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *chloroform* and 25 volumes of *methanol*.

Solvent mixture. 80 volumes of *chloroform* and 20 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.125 g of Clozapine and dissolve in 25.0 ml of the solvent mixture.

Reference solution (a). A 0.5 per cent w/v solution of *clozapine IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 200.0 ml with the solvent mixture.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 250.0 ml with the solvent mixture.

Reference solution (d). Dilute 3.0 ml of reference solution (a) to 1000.0 ml with the solvent mixture.

Reference solution (e). Dilute 1.0 ml of reference solution (a) to 500.0 ml with the solvent mixture.

Reference solution (f). Dilute 1.0 ml of reference solution (a) to 1000.0 ml with the solvent mixture.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Compare the intensities of any secondary spots observed in the chromatogram obtained with the test solution with those of the principal spots in the chromatogram obtained with the reference solutions : no secondary spot from the chromatogram obtained with the test solution is larger or more intense than the principal spot obtained with reference solution (b) (0.5 per cent); and the sum of the intensities of all secondary spots obtained with the test solution corresponds to not more than 2.0 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.125 g of Clozapine, dissolve in 640 ml of *methanol* and add sufficient *water* to produce 1000 ml.

Reference solution (a). Weigh 12.5 mg of *clozapine IPRS* in 80 ml of *methanol* and dilute to 100.0 ml with *water*.

Reference solution (b). Weigh accurately about 10 mg of Clozapine, add 5 ml of *0.1 M hydrochloric acid* and heat for 2 hours at 90°. Cool, add 15 ml of *water*, dilute with *methanol* to 100.0 ml and mix. To 10.0 ml of the solution add 10.0 ml of reference solution (a) and mix.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: 800 volumes of *methanol*, 200 volumes of *water* and 0.75 volumes of *triethylamine*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 257 nm,
- injection volume: 10 µl.

Inject the reference solution (a). The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

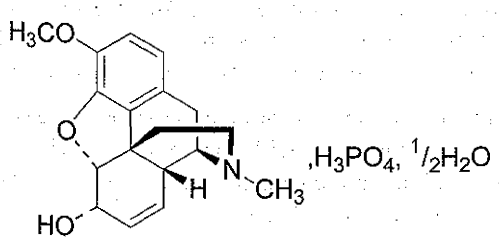
Inject reference solution (b). The resolution between the clozapine peak and any secondary peak is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{19}ClN_4$ in the tablets.

Codeine Phosphate

Codeine Phosphate Hemihydrate



$C_{18}H_{21}NO_3,H_3PO_4,½\,H_2O$ Mol. Wt. 406.4

Codeine Phosphate is *(5R,6S)*-7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6-ol dihydrogen phosphate hemihydrate, an alkaloid occurring in *Papaver somniferum*.

Codeine Phosphate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{21}NO_3,H_3PO_4$, calculated on the dried basis.

Category. Analgesic; antidiarrhoeal; cough suppressant.

Description. Colourless crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D, E and F are carried out. Tests B, C, D and E may be omitted if tests A and F are carried out.

A. Dissolve 0.2 g in 4 ml of *water*, add 2 ml of *2 M sodium hydroxide* and induce crystallisation, if necessary by scratching the wall of the tube with a glass rod and cooling in ice. The residue after washing with *water* and drying at 100° to 105° complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *codeine phosphate IPRS* treated in the same manner or with the reference spectrum of codeine.

B. To 25 ml of a 0.04 per cent w/v solution add 25 ml of *water* and 10 ml of *1 M sodium hydroxide* and dilute to 100 ml of *water*. When examined in the range 230 nm to 360 nm, the resulting solution shows an absorption maximum at 284 nm, about 0.38 (2.4.7).

C. On the surface of one drop of *nitric acid* place a little of the powder; a yellow but not red colour is produced (distinction from morphine).

D. Dissolve 0.1 g in 1 ml of *sulphuric acid* and 1 drop of *ferric chloride test solution* and warm gently; a bluish violet colour is produced. Add 1 drop of *dilute nitric acid*; the colour changes to red.

E. It gives the reaction of alkaloids (2.3.1).

F. It gives reaction (A) of phosphates (2.3.1).

Tests

Appearance of solution. A 4.0 per cent w/v solution in *carbon dioxide-free water* prepared from *distilled water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 4.2 to 5.0, determined in a 4.0 per cent w/v solution.

Specific optical rotation (2.4.22). – 102° to – 98.0° to, determined in a 2.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g each of the substance under examination and *sodium octanesulphonate* in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *codeine impurity A IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). To 0.25 ml of the test solution, add 2.5 ml of reference solution (a).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 1.08 g of *sodium octanesulphonate* in a mixture of 20 ml of *glacial acetic acid* and 250 ml of *acetonitrile* and dilute to 1000 ml with *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Codeine impurity B[1]	0.7	—
Codeine impurity E[2]	0.7	—
Codeine (Retention time: about 6 minutes)	1.0	—
Codeine impurity A[3]	2.0	—
Codeine impurity C[4]	2.3	0.25
Codeine impurity D[5]	3.6	—

[1]morphine,

[2]7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,10-diol,

[3]Methylcodeine,

[4] Codeine dimer,

[5]3-*O*-(codein-2-yl)morphine.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to codeine and codeine impurity A is not less than 3.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 10 times the retention time of codeine peak. In the chromatogram obtained with the test solution, the area of peak corresponding to codeine impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). The sum of areas of the peak corresponding to codeine impurities B and E is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent). The area of peak corresponding to codeine impurities C and D is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks other than codeine impurity A is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphates (2.3.17). 7.5 ml of a 2.0 per cent w/v solution in *distilled water* complies with the limit test for sulphates (0.1 per cent).

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.4 g of the substance under examination and dissolve in a mixture of 10 ml of *anhydrous glacial acetic acid* and 20 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, using a few drops of crystal violet solution as indicator. Carry out blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03974 g of $C_{18}H_{21}NO_3,H_3PO_4$.

Storage. Store protected from light.

Codeine Syrup

Codeine Phosphate Syrup

Codeine Syrup is a 0.5 per cent w/v solution of Codeine Phosphate in a suitable flavoured vehicle.

Codeine Syrup contains not less than 0.48 per cent w/v and not more than 0.52 per cent w/v of codeine phosphate, $C_{18}H_{21}NO_3,H_3PO_4,½\,H_2O$.

Identification

To 10 ml of the syrup add sufficient *dilute ammonia solution* until the solution is alkaline and extract with three quantities, each of 10 ml, of *chloroform*. Evaporate the combined chloroform extracts to dryness on a water-bath and dry the residue at 80°. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *codeine phosphate IPRS* treated in the same manner or with the reference spectrum of codeine.

B. Dissolve 10 mg in 1 ml of *sulphuric acid* and 1 drop of *ferric chloride test solution* and warm gently; a bluish violet colour is produced. Add 1 drop of *dilute nitric acid*; the colour changes to red.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 72 volumes of *ethanol*, 30 volumes of *cyclohexane* and 6 volumes of *strong ammonia solution.*

Test solution. To 20 ml of the syrup add 20 ml of *water* and 2 ml of *strong ammonia solution* and extract with two quantities, each of 20 ml, of *chloroform.* Dry the combined extracts with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform.*

Reference solution (a). Dilute 1.5 volumes of the test solution to 100 volumes with the same solvent.

Reference solution (b). Dilute 1 volume of the test solution to 100 volumes with the same solvent.

Apply separately to the plate 10 µl of each solution. After development, dry the plate in air and spray with *acidic potassium iodobismuthate solution.* Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot, with an R_f value higher than that of the principal spot, is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Weigh accurately about 10.0 g, add *dilute ammonia solution* until the solution is alkaline to *litmus paper* and extract with four quantities, each of 25 ml, of *chloroform.* Wash each extract successively with the same 10 ml of *water*; combine the chloroform extracts and evaporate to dryness on a water-bath. To the residue add 5 ml of *ethanol (95 per cent)* and again evaporate to dryness. Dissolve the residue in 5.0 ml of *0.05 M hydrochloric acid* and titrate the excess of acid with *0.05 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.05 M hydrochloric acid* is equivalent to 0.02032 g of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$.

Determine the weight per ml of the syrup (2.4.29) and calculate the content of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$, weight in volume.

Storage. Store protected from light.

Codeine Tablets

Codeine Phosphate Tablets

Codeine Phosphate Tablets contain Codeine Phosphate Hemihydrate.

Codeine Phosphate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of codeine phosphate hemihydrate, $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$.

Usual strength. 10 mg.

Identification

Macerate a quantity of the powdered tablets containing 50 mg of Codeine Phosphate with 5 ml of *1 M sulphuric acid* and 15 ml of *water*. Filter, make alkaline with *5 M ammonia*, extract with successive quantities of *chloroform* and evaporate the combined chloroform extracts to dryness on a water-bath. The residue complies with the following tests.

A. Place a few mg of residue on the surface of a drop of *nitric acid.* A yellow but no red colour is produced (distinction from morphine).

B. Dissolve 10 mg in 1 ml of *sulphuric acid* and 1 drop of *ferric chloride test solution* and warm gently; a bluish violet colour is produced. Add 1 drop of *dilute nitric acid*; the colour changes to red.

C. Dissolve a quantity of powdered tablets containing 20 mg of codeine phosphate in *water* and filter. Filtrate gives reaction (A) of phosphates (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 284 nm (2.4.7). Calculate the content of $C_{18}H_{21}NO_3.H_3PO_4,½H_2O$ in the medium from the absorbance obtained from a solution of known concentration of *codeine phosphate IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{18}H_{21}NO_3.H_3PO_4,½H_2O$.

Foreign alkaloids. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 72 volumes of *ethanol*, 30 volumes of *cyclohexane* and 6 volumes of *strong ammonia solution.*

Test solution. Shake a quantity of the powdered tablets containing 0.25 g of Codeine Phosphate with 10 ml of a mixture of 4 volumes of *0.01 M hydrochloric acid* and 1 volume of *ethanol* for 15 minutes and filter.

Reference solution (a). Dilute 1.5 ml of the test solution to 100.0 ml with *0.01 M hydrochloric acid.*

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *0.01 M hydrochloric acid.*

Apply separately to the plate 20 µl of each solution. After development, dry the plate in air and spray with *acidic potassium iodobismuthate solution.* Any secondary spot in

the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.5 per cent) and not more than one such spot, with an R_f value higher than that of the principal spot, is more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent).

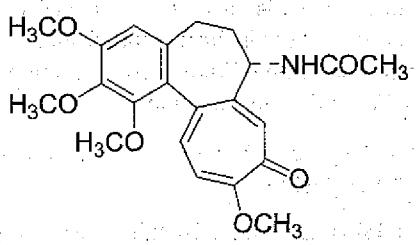
Other tests. Comply with the tests stated under Tablets.

Assay. Disperse a quantity of the powdered tablets containing 0.3 g of Codeine Phosphate, dissolve in 20 ml of *0.25 M sulphuric acid*, filter and wash the residue with *0.25 M sulphuric acid*. Make alkaline with *5 M ammonia* and extract with successive quantities of *chloroform*. Wash each *chloroform solution* with 10 ml of *water* and evaporate the chloroform. To the residue add 5 ml of *ethanol (95 per cent)* and again evaporate to dryness. Dissolve the residue in 1 ml of neutralised *ethanol (95 per cent)*, add 10.0 ml of *0.1 M hydrochloric acid* and 10 ml of *water*. Titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.04064 g of $C_{18}H_{21}NO_3,H_3PO_4,½H_2O$.

Storage. Store protected from light.

Colchicine



$C_{22}H_{25}NO_6$ Mol. Wt. 399.4

Colchicine is *N*-[(7*S*, 12a*M*)-1, 2, 3, 10-tetramethoxy-9-oxo-5, 6, 7, 9-tetrahydrobenzo[*a*]heptalen-7-yl] acetamide, an alkaloid which occurs in the corm and seeds of various species of *Colchicum*.

Colchicine contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{22}H_{25}NO_6$, calculated on the anhydrous and solvent free basis.

NOTE — Colchicine is extremely poisonous, handle with caution.

Category. Gout suppressant.

Description. A pale yellow, amorphous or crystalline powder.

Identification

Tests A, C and D may be omitted if test B, is carried out. Test B may be omitted if tests A, C and D are carried out.

A. When examined in the range 230 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima, at about 243 nm and 350 nm and the absorbance ratio of A_{243}/A_{350} is 1.7 to 1.9.

B. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *colchicine IPRS* or with the reference spectrum of colchicine. Ignore any peak at 1735 cm^{-1}.

C. To 0.5 ml of solution A (see Test), add 0.5 ml of *dilute hydrochloric acid* and 0.15 ml of a 10.5 per cent w/v solution of *ferric chloride hexahydrate*. The solution is yellow and becomes dark green on boiling for 30 seconds. Cool, add 2 ml of *methylene chloride* and shake. The organic layer is greenish-yellow.

D. Dissolve 30 mg in 1.0 ml of *ethanol (95 per cent)* and add 0.15 ml of a 10.5 per cent w/v solution of *ferric chloride hexahydrate*. A brownish red colour develops.

Tests

Appearance of solution. A 0.5 per cent w/v solution in *carbon dioxide-free water* (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution GYS3 (2.4.1).

Acidity or alkalinity. To 10 ml of solution A, add 0.1 ml of *bromothymol blue solution*; either the solution does not change colour or it becomes green. Not more than 0.1 ml of *0.01 M sodiumhydroxide* is required to change the colour of the indicator to blue.

Specific optical rotation (2.4.22). –250° to –235°, determined in a 0.5 per cent w/v solution in *ethanol (95 per cent)* at 20°.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water*.

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a). Dissolve 5 mg of *colchicine for peak identification IPRS* (containing impurities A, E and G) in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Reference solution (b). A 0.001 per cent w/v solution of *colchicine IPRS* in the solvent mixture.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 450 volumes of a 0.68 per cent w/v solution of *potassium dihydrogen orthophosphate* and 530 volumes of *methanol,* cool to room temperature and dilute to 1000 ml with *methanol,* adjusted to the pH 5.5 with *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Name	Relative retention time
Colchicine impurity E[1]	0.6
Colchicine impurity B[2]	0.9
Colchicine impurity A[3]	0.94
Colchicine (Retention time: about 7 minutes)	1.0
Colchicine impurity G[4]	1.4

[1]3-*O*-demethylcolchicine,
[2]conformational isomer,
[3]*N*-deacetyl-*N*-formylcolchicine,
[4]Y-lumicolchicine.

Inject reference solution (a). Adjust the sensitivity of the system so that the peak-to-valley ratio is minimum 2.0, where H_p is height above the baseline of the peak due to impurity A and H_V is the height above the baseline of the lowest point of the curve separating this peak from the peak due to colchicine, the peak-to-valley ratio is minimum 2.0, where H_p is height above the baseline of the peak due to impurity B and H_V is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A.

Inject reference solution (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. The area of any peak corresponding to colchicine impurity A is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent), the area of any peak corresponding to colchicine impurity G multiplied with correction factor 1.6, is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent), the area of any peak corresponding to colchicine impurity E is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) and peak due to colchicine impurity B.

Impurity F (Colchiceine). Not more than 0.2 per cent.

Dissolve 50 mg in 5 ml of *water,* add 0.1 ml of a 10.5 per cent w/v solution of *ferric chloride hexahydrate.* Any colour produced is not more than intense than that obtained by mixing 2.0 ml of *ferric chloride colorimetric solution (FCS)* with 1.0 ml of *cobaltous chloride colorimetric solution (CCS)* and 2.0 ml of *cupric sulphate colorimetric solution (CSS)* (2.4.1).

Ethyl acetate. Not more than 8.0 per cent w/w.

Determine by gas chromatography (2.4.13).

Internal standard solution. Dilute 0.5 ml of *n-propyl alcohol* to 100.0 ml with *water.*

Test solution. Dissolve 0.25g of the substance under examination in 8 ml of *water,* add 1.0 ml of the internal standard solution and dilute to 10.0 ml with *water.*

Reference solution. A 0.09 per cent w/v solution of *ethyl acetate* prepared by mixing 1.0 ml of *ethyl acetate,* 0.5 ml of *diacetone alcohol,* and 0.5 ml of *n-propyl alcohol* and diluted to 1000.0 ml with *water.*

Chromatographic system
- a fused silica column 30 m x 0.53 mm, packed with polyethylene glycol 20 M (film thickness 1.0 µm),
- temperature:
  column.40°, hold for 20 minutes, 40° to 200° @ 20°per minute and hold for 10 minutes,
- inlet port 180° and detector at 220°,
- split ratio: 15:1,
- flame ionization detector,
- flow rate: 5.72 ml per minute using nitrogen as the carrier gas,
- injection volume: 2 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation of peak area ratio of ethyl acetate to n-propyl alcohol and diacetone alcohol to n-propyl alcohol peak, from replicate injections is not more than 15.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of ethyl acetate.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light and prepare the solutions immediately before use.

Solvent mixture. Equal volumes of *methanol* and *water.*

Test solution. Dissolve 30 mg of Colchicine in about 50 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 50.0 ml with the solvent mixture and filter.

Reference solution. A 0.0006 per cent w/v solution of *colchicine IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5µm),
- mobile phase: dilute 45 ml of *0.5 M monobasic potassium phosphate* to 450 ml, with *water* add about 530 ml of *methanol,* cool to room temperature and dilute to 1000 ml with *methanol,* adjusted to pH 5.5 with *0.5 M orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4500 theoretical plates and the relative standard deviation for replicate injections is not more than 2 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{25}NO_6$.

Storage. Store protected from light and moisture.

Colchicine Tablets

Colchicine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of colchicine, $C_{22}H_{25}NO_6$

Usual strengths. 250 µg; 500 µg.

Identification

A. Disperse a quantity of the powdered tablets containing 5 mg of Colchicine in 50 ml of *methanol (50 per cent)* and filter. Dilute 10 ml of the filtrate to 100 ml with *methanol (50 per cent).* When examined the solution in the range 220 nm to 400 nm (2.4.7), shows absorption maxima at about 246 nm and 352 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of a buffer solution pH 6.8 prepared by mixing 3.52 g of *sodium dihydrogen orthophosphate monohydrate* and 4.35 g of *disodium hydrogen orthophosphate dihydrate* in sufficient *water* to produce 1000 ml,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), using chromatographic system, as described under Related substances with 50 µl injection volume and spectrophotometer set at 243 nm.

NOTE — Carry out the test protected from light.

Test solution. Dilute the filtrate if necessary, with the dissolution medium to obtain a solution containing 0.0001 per cent w/v of Colchicine.

Reference solution. A 0.0001 per cent w/v solution of *colchicine IPRS* in the dissolution medium.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{25}NO_6$ in the medium

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{25}NO_6$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light.

Test solution. Disperse a quantity of powdered tablets containing 5 mg of Colchicine in 40 ml of *methanol (50 per cent)* with the aid of ultrasound and dilute to 50.0 ml with the same solvent, filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol (50 per cent).*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with *methanol (50 per cent).*

Reference solution (c). A 0.1 per cent w/v solution of *colchicine for system suitability A IPRS* in *methanol (50 per cent).*

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Lichrosorb RP 8),
- mobile phase: A. *water,*
  B. *methanol,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	52	48
12	52	48
25	20	80
30	20	80
32	52	48
38	52	48

The relative retention time with reference to colchicine (retention time: about 13 minutes) for impurity A is about 0.9.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to colchicine impurity A and colchicine peak is not less than 1.5.

Inject the test solution, reference solution (a) and (b). In the chromatogram obtained with the test solution, the area of any peak corresponding to colchicine impurity A is not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications..

Test solution. Disperse one tablet in 4 ml of the solvent mixture, with the aid of ultrasound and dilute to 5.0 ml with the solvent mixture, filter.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{25}NO_6$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light.

Solvent mixture. Equal volumes of *methanol* and *water.*

Test solution. Disperse 10 intact tablets in 40 ml of the solvent mixture, with the aid of ultrasound and dilute to 50.0 ml with the solvent mixture, filter.

Reference solution (a). Dissolve quantity of *colchicine IPRS* in the solvent mixture and dilute with the solvent mixture to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Reference solution (b). A 0.1 per cent w/v solution of *colchicine for system suitability A IPRS* in the solvent mixture.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to colchicine impurity A and colchicine is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{25}NO_6$ in the tablets.

Storage. Store protected from light.

Colchicine and Probenecid Tablets

Colchicine and Probenecid Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of colchicine, $C_{22}H_{25}NO_6$, and not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of probenecid, $C_{13}H_{19}NO_4S$.

Usual strength. Colchicine, 1 mg and Probenecid, 250 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution.*

Test solution (a). Shake a quantity of the powdered tablets containing 1 mg of Colchicine with 15 ml of *water*, mix, filter, extract the filtrate with 25 ml of *chloroform* and evaporate the chloroform extract to a volume of about 1 ml.

Test solution (b). Shake a quantity of the powdered tablets containing 10 mg of Probenecid with 10 ml of *chloroform,* allow to settle and decant the clear supernatant liquid.

Reference solution (a). A solution containing 0.1 per cent w/v of *colchicine IPRS.*

Reference solution (b). A solution containing 0.1 per cent w/v of *probenecid IPRS.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (a) corresponds to that in the chromatogram obtained with reference solution (a). Similarly, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

Uniformity of content. *For colchicine* — Complies with the test stated under Tablets using the method described under Assay.

Other tests. Comply with the tests stated under Tablets.

Assay. *For colchicine — Carry out the determination without delay, under subdued light, using low actinic glassware.*

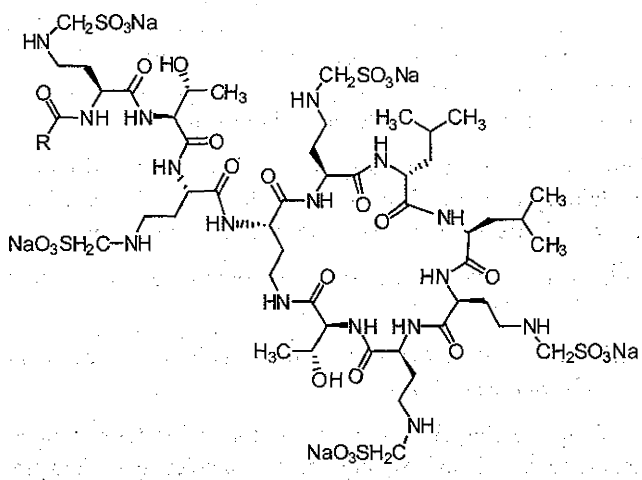
Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 1 mg of Colchicine, shake with 75 ml of *ethanolic sodium carbonate* for 30 minutes, add sufficient of *ethanolic sodium carbonate* to produce 100.0 ml and filter. Measure the absorbance of the resulting solution at the maximum at about

350 nm (2.4.7). Calculate the content of $C_{22}H_{25}NO_6$ taking 440 as the specific absorbance at 350 nm.

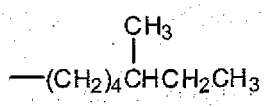
For probenecid — Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.25 g of Probenecid, shake with 100 ml of *0.1 M sodium hydroxide* for 10 minutes, add sufficient of *0.1 M sodium hydroxide* to produce 250.0 ml, mix and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *0.1 M sodium hydroxide*. Dilute 10.0 ml to 50.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 248 nm (2.4.7). Calculate the content of $C_{13}H_{19}NO_4S$ taking 332 as the specific absorbance at 248 nm.

Storage. Store protected from light.

Colistimethate Sodium

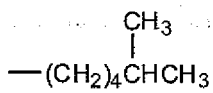


Colistin A component R =



$C_{58}H_{105}N_{16}Na_5O_{28}S_5$ Mol. Wt.: 1749.8

Colistin B component R =



$C_{57}H_{103}N_{16}Na_5O_{28}S_5$ Mol. Wt.: 1735.8

Colistimethate Sodium is prepared from colistin by the action of formaldehyde and sodium hydrogen sulphite.

Semi-synthetic product derived from a fermentation product.

Colistimethate Sodium contains not less than 11500 Units per mg, calculated on the dried basis.

Category. Antibiotic.

Description. A white or almost white, hygroscopic powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

NOTE — Protect the solutions from light.

Mobile phase. A mixture of 25 volume of *water* and 75 volumes of *phenol*.

Test solution. Dissolve 5 mg of the substance under examination in 1.0 ml of a mixture of equal volumes of *hydrochloric acid* and *water*, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water-bath, continue to heat until the *hydrochloric acid* has evaporated and dissolve the residue in 0.5 ml of *water*.

Reference solution (a). A 0.2 per cent w/v solution of *leucine* in *water*.

Reference solution (b). A 0.2 per cent w/v solution of *threonine* in *water*.

Reference solution (c). A 0.2 per cent w/v solution of *phenylalanine* in *water*.

Reference solution (d). A 0.2 per cent w/v solution of *serine* in *water*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 105°, spray with *ethanolic ninhydrin solution* and heat at 110° for 5 minutes. The spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution (a) and (b), but shows no spots corresponding to those in the chromatograms obtained with reference solution (c) and (d). It also shows a spot with a very low Rf value (2,4-diaminobutyric acid).

B. Dissolve 5 mg in 3 ml of *water*, add 3 ml of *dilute sodium hydroxide solution*. Shake and add 0.5 ml of a 1.0 per cent w/v solution of *copper sulphate;* a violet colour is produced.

C. Dissolve 50 mg in 1 ml of *1 M hydrochloric acid*, add 0.5 ml of *0.01 M iodine*. The solution is decolourised and gives the reaction (a) of sulphates (2.3.1).

D. It gives reaction (b) of sodium salts (2.3.1).

Tests

Appearance of solution. A 1.6 per cent w/v solution in *water* is clear (2.4.1).

pH (2.4.24). 6.5 to 8.5, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*, measure after 30 minutes.

Specific optical rotation (2.4.22). −51.0° to −46.0°, determined in a 5.0 per cent w/v solution in *water*.

Free colistin. Dissolve 80 mg in 3 ml of *water*, add 0.1 ml of a 10.0 per cent w/v solution of *silicotungstic acid*; after 10 to 20 seconds, the solution is not more opalescent than opalescence standard OS2 (2.4.1).

Sulphated ash (2.3.18). 16.0 per cent to 21.0 per cent, determined on 0.5 g.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° over *diphosphorus pentoxide* at a pressure not exceeding 0.67 kPa for 3 hours.

Colistimethate sodium intended for use in the manufacture of parenteral preparation without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 2.0 Endotoxin Units per mg of colistin.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

Storage. Store protected from light and moisture.

Colistimethate Injection

Colistimethate Injection is a sterile solution of Colistimethate Sodium in Sodium Chloride Intravenous Infusion. It is prepared by dissolving Colistimethate Sodium for Injection in the requisite amount of Sodium Chloride Intravenous Infusion.

Storage. Colistimethate Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

Colistimethate Sodium for Injection

Colistimethate Sodium for Injection is a sterile material consisting of Colistimethate Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Colistimethate Sodium for Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of colistimethate sodium.

Usual strengths. 1 million IU and 2 million IU per vial.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

NOTE—Protect the solutions from light.

Mobile phase. A mixture of 25 volume of *water* and 75 volumes of *phenol*.

Test solution. Dissolve a quantity containing 62,500 Units of Colistimethate Sodium in 1 ml of a mixture of equal volumes of *hydrochloric acid* and *water*, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water-bath, continue to heat until the *hydrochloric acid* has evaporated and dissolve the residue in 0.5 ml of *water*.

Reference solution (a). A 0.2 per cent w/v solution of *leucine* in *water*.

Reference solution (b). A 0.2 per cent w/v solution of *threonine* in *water*.

Reference solution (c). A 0.2 per cent w/v solution of *phenylalanine* in *water*.

Reference solution (d). A 0.2 per cent w/v solution of *serine* in *water*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 105°, spray with *ethanolic ninhydrin solution* and heat at 110° for 5 minutes. The spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution (a) and (b), but shows no spots corresponding to those in the chromatograms obtained with reference solution (c) and (d). It also shows a spot with a very low R_f value (2,4-diaminobutyric acid).

B. Dissolve a quantity containing 125,000 IU in 5 ml of *water*. Heat 0.5 ml of the solution with 0.5 ml of *chromotropic acid–sulphuric acid solution* at 100° for 30 minutes; a purple colour is produced (distinction from colistin sulphate).

C. Dissolve a quantity containing 625,000 IU in 1 ml of *1 M hydrochloric acid*, add 0.5 ml of *0.01 M iodine*. The solution is decolourised and gives the reaction (a) of sulphates (2.3.1).

D. It gives the reaction (b) of sodium salts (2.3.1).

Tests

pH (2.4.24). Dissolve a quantity in *carbon dioxide-free water* to obtain a solution containing 125,000 IU per ml. The pH of the solution, measured 30 minutes after preparation, is 6.5 to 8.5.

Free colistin. Dissolve a quantity containing 1,000,000 IU in 3 ml of *water*, add 0.1 ml of a 10.0 per cent w/v solution of *silicotungstic acid* and allow to stand for 10 to 20 seconds. The solution is not more opalescent than opalescence standard OS2 (2.4.1).

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° over *diphosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 3 hours.

Colistimethate Sodium intended for use in the manufacture of parenteral preparation without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 43.75 Endotoxin Unit per ml of a solution containing 250,000 IU per ml in Water for Injections.

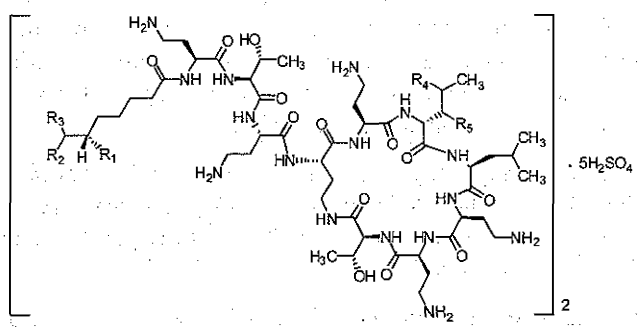
Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Mix the contents of the 10 containers and carry out the microbiological assay of antibiotics (2.2.10).

Storage. Store protected from moisture.

Labelling. The label of the sealed container states the total number of IU (units) contained in it.

Colistin Sulphate



Polymyxin	R1	R2	R3	R4	R5	Mol. Formula	M_r
E1	CH_3	CH_3	H	CH_3	H	$C_{53}H_{100}N_{15}O_{13}$	1170
E2	CH_3	H	H	CH_3	H	$C_{52}H_{98}N_{15}O_{13}$	1155
E3	H	CH_3	H	CH_3	H	$C_{52}H_{98}N_{15}O_{13}$	1155
E1-I	CH_3	CH_3	H	H	CH_3	$C_{53}H_{100}N_{15}O_{13}$	1170
E1-7MOA	H	CH_3	CH_3	CH_3	H	$C_{53}H_{100}N_{15}O_{13}$	1170

Category. Antibacterial.

Colistin Sulphate is a mixture of the sulphates of polypeptides produced by the growth of certain strains of *Bacillus polymyxa* var. *colistinus* or by any other means.

Colistin Sulphate contains not less than 77.0 per cent sum of *polymyxins E1, E2, E3, E1-I* and *E1-7MOA*, and not more than 10.0 per cent each of *polymyxins E1-I, E1-7MOA* and *polymyxin E3*, calculated on the dried basis.

Description. A white or almost white powder; hygroscopic.

Identification

Test A, C and D may be omitted if tests B and E are carried out. Tests B may be omitted if test A, C, D and E are carried out.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

NOTE — Carry out the test protected from light.

Mobile phase. A mixture of 75 volumes of *phenol* and 25 volumes of *water*.

Test solution. Dissolve 5 mg of the substance under examination in 1 ml of equal volume of *hydrochloric acid* and *water*, heat at 135° for 5 hours in a sealed tube. Evaporate to dryness on a water-bath and continue the heating until the *litmus paper* has turned red and dissolve the residue in 0.5 ml of *water*.

Reference solution (a). A 0.2 per cent w/v solution of *leucine IPRS* in *water*.

Reference solution (b). A 0.2 per cent w/v solution of *threonine IPRS* in *water*.

Reference solution (c). A 0.2 per cent w/v solution of *phenylalanine IPRS* in *water*.

Reference solution (d). A 0.2 per cent w/v solution of *serine IPRS* in *water*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. After development, dry the plate at 105°, spray with *ninhydrin solution* and heat at 110° for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatograms obtained with reference solution (a) and (b), but shows no spots corresponding to those in the chromatograms obtained with reference solution (c) and (d); the spot obtained with the test solution also shows a spot with a very low R_f value of *2, 4-diaminobutyric acid*.

B. In the Assay, the peaks due to *polymyxin E1* and *polymyxin E2* in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with reference solution (a).

C. Dissolve 5 mg in 3 ml of *water*, add 3 ml of *dilute sodium hydroxide solution*. Shake and add 0.5 ml of a 1.0 per cent solution of *copper sulphate;* A violet colour is produced.

D. Dissolve 50 mg in 1 ml of *1 M hydrochloric acid*; add 0.5 ml of *0.01 M iodine*. The solution remains colored.

E. It gives reaction (A) of sulphates (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.0, determined in 1.0 per cent w/v solution in *carbon dioxide-free water.*

Specific optical rotation (2.4.22). –73° to –63° determined in a 5.0 per cent w/v solution in *water.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 40 ml of *water* and dilute to 50.0 ml with *acetonitrile.*

Reference solution (a). Dissolve 25 mg of *colistin sulphate IPRS* in 40 ml of *water* and dilute to 50.0 ml with *acetonitrile.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with a mixture of 20 volumes of *acetonitrile* and 80 volumes of *water.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 30°,
- mobile phase: a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution prepared by dissolving 4.46 g of *anhydrous sodium sulphate* in 900 ml of *water*, adjusted to pH 2.4 with *dilute phosphoric acid* and dilute to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Name	Relative retention time
polymyxin E2	0.45
polymyxin E3	0.5
polymyxin E1-1	0.8
polymyxin E1(Retention time about 16 minutes)	1.0
polymyxin E1-7MOA	1.1

Inject reference solution (a). Run the chromatogram 1.5 times the retention time of polymyxin E1.

The test is not valid unless the resolution between the peak due to polymyxin E2 and polymyxin E1 is not less than 8.0, between the peaks due to polymyxin E2 and polymyxin E1-I is not less than 6.0, between the peaks due to polymyxin E1-I and polymyxin E1 is not less than 2.5 and between the peaks due to polymyxin E1 and polymyxin E1-7MOA is not less than 1.5.

Inject reference solution (b) and the test solution. The area of any peak other than principal peak in the chromatogram obtained with test solution is not greater than 4.0 per cent and sum of all the peaks is not greater than 23.0 per cent. Ignore any peak with area due to polymyxin E1 in the chromatogram obtained with reference solution (b); and polymyxins E2, E3, E1-I, E1 and E1-7MOA.

Sulphates. 16.0 to 18.0 per cent calculated on dried basis, determined by following method.

Dissolve 0.25 g in 100 ml of *distilled water* and adjusted to pH 11 with *concentrated ammonia*. Add 10.0 ml of *0.1 M barium chloride* and 0.5 mg of *phthalein purple* as an indicator and titrate with *0.1 M sodium edetate*, when the colour of the solution begins to change add 50 ml *ethanol (95 per cent)* and continuing the titration until the violet-blue colour disappears. Perform the blank determination and make any necessary correction.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of SO_4.

Sulphated ash (2.3.18). Not more than 1.0 per cent, determined on 1.0 g.

Loss on drying (2.4.19). Not more than 3.5 per cent, determined on 1.0 g by drying in an oven at 60° over *diphosphorus pentoxide* at a pressure not exceeding 670 Pa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 40 ml of *water* and dilute to 50.0 ml with *acetonitrile.*

Reference solution. Dissolve 25 mg of *colistin sulphate IPRS* in 40 ml of *water* and dilute to 50.0 ml with *acetonitrile.*

Use the Chromatographic system as described under Related substances.

Inject the reference solution and the test solution.

Calculate the percentage content of polymyxin E3, of polymyxin E1-I, of polymyxin E1-7MOA, and the sum of polymyxins E1, E2, E3, E1-I and E1-7MOA. Using the following expression.

$$C_{Ei} = \frac{A_{Ei} \times m_2 \times D_{Ei}}{m_1 \times B_{Ei}}$$

where, C_{EI} = percentage content of polymyxin *Ei*,

A_{EI} = area of the peak due to polymyxin *Ei* in the chromatogram obtained with the test solution,

m_1 = mass in milligrams of the substance to be examined (dried substance) in the test solution,

B_{EI} = area of the peak due to polymyxin *E1* in the chromatogram obtained with reference solution (a),

m_2 = mass in milligrams of *colistin sulphate IPRS* in reference solution (a),

D_{EI} = declared percentage content for polymyxin *E1* in *colistin sulphate IPRS*.

Storage. Store protected from light and moisture.

Colistin Sulphate Oral Suspension

Colistin Sulphate Oral Suspension is a dry mixture of Colistin Sulphate with or without one or more suitable buffers, colours, diluents, dispersants, and flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of Water just before use.

Colistin Sulphate Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of colistin.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of colistin.

Tests

pH (2.4.24). 5.0 to 6.0 in the constituted suspension.

Other tests. Comply with the tests stated under Oral Liquids.

Loss on drying (2.4.19). Not more than 3.0 per cent determined on 0.1 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.67 kPa.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the result in mg of Colistin per ml.

Storage. Store protected from light and moisture.

Colistin Tablets

Colistin Sulphate Tablets

Colistin Tablets contain not less than 77.0 per cent contain sum of *polymyxins E1, E2, E3, E1-I* and *E1-7MOA* of the stated amount of Colistin sulphate, and not more than 10.0 per cent of each *polymyxins E1-I, E1-7MOA* and *polymyxin E3*.

Usual strengths. 1000,000 Units (equivalent to 80 mg).

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

NOTE—Carry out the test protected from light.

Mobile phase. A mixture of 75 volumes of *phenol* and 25 volumes of *water*.

Test solution. Shake a quantity of powdered tablets containing 10 mg of Colistin Sulphate with 10 ml of *water* and filter, add 0.5 ml of *hydrochloric acid* to 0.5 ml of the filtrate, heat in a sealed tube at 135° for 5 hours, evaporate to dryness on a water bath, continue to heat until any residual hydrogen chloride has been removed. Dissolve the residue in 0.5 ml of *water* and centrifuge, if necessary.

Reference solution (a). A 0.25 per cent w/v solution of L-*leucine IPRS* in *water*.

Reference solution (b). A 0.25 per cent w/v solution of L-*threonine IPRS* in *water*.

Reference solution (c). A 0.25 per cent w/v solution of L- *phenylalanine IPRS* in *water*.

Reference solution (d). A 0.25 per cent w/v solution of L-*serine IPRS* in *water*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. After development, dry the plate at 105°, spray with *ninhydrin solution* and heat at 110° for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatograms obtained with reference solution (a) and (b), but shows no spots corresponding to those in the chromatograms obtained with reference solution (c) and (d); the spot obtained with the test solution also shows a spot with a very low R_f value of *2, 4-diaminobutyric acid*.

B. Heat 0.5 ml of the filtrate with 0.5 ml of *chromotropic acid-sulphuric acid solution* at 100° for 30 minutes. No purple colour is produced (distinction from colistin sulfomethate).

C. The filtrate gives reaction (A) of *sulphates* (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 25 mg of Colistin Sulphate for 20 minutes with 40 ml of *water*, dilute to 50.0 ml with *acetonitrile* and filter.

Reference solution (a). Dissolve 25 mg of *colistin sulphates IPRS*, add 40 ml of *water* and dilute to 50 ml with *acetonitrile*.

Reference solution (b). Dilute 1 volume of reference solution (a) to 100 volumes with a mixture of 20 volumes of *acetonitrile* and 80 volumes of *water*.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (3.5 µm).
- column temperature: 30°,
- mobile phase: a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution prepared by dissolving 4.46 g of *anhydrous sodium sulphate* in 900 ml of *water*, adjusted to pH 2.4 with *dilute orthophosphoric acid* and dilute to 1000 ml with *water*.
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject reference solution (a). Run the chromatogram 1.5 times the retention time of polymyxin E1. The relative retention time with reference to polymyxin E1 is about 16, for polymyxin E2 is about 0.45, for polymyxin E3 is about 0.5, for polymyxin E1-I is about 0.8 and polymyxin E1-7MOA is about 1.1.

The test is not valid unless the resolution between the peak due to polymyxin E2 and polymyxin E1 is not less than 8.0; between the peaks due to polymyxin E2 and polymyxin E1-I is not less than 6.0; between the peaks due to polymyxin E1-I and polymyxin E1 is not less than 2.5 and between the peaks due to polymyxin E1 and polymyxin E1-7MOA is not less than 1.5.

Inject reference solution (b) and the test solution. The area of any peak other than principal peak in the chromatogram obtained with test solution is not greater than 4.0 per cent and sum of all the peaks is not greater than 23.0 per cent. Ignore any peak with area less than the area of peak due to polymyxin E1 in the chromatogram obtained with reference solution (b) and any peak due to polymyxin E2, E3, E1-I, E1 and E1-7MOA.

Other tests. Comply with the test stated under Tablets.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10).

The precision of the assay is such that the fiducial limits of error are not less than 95 per cent and not more than 105 per cent of the estimated potency. The upper fiducial limit of error is not less than 97.0 per cent and the lower fiducial limit of error is not more than 110.0 per cent of the stated number of units.

Storage. Store protected from light.

Corn Oil

Corn Oil is the refined fixed oil obtained from the embryo of *Zea mays* Linne (Fam. Gramineae).

Category. Pharmaceutical aid.

Description. A clear, light yellow-coloured, oily liquid with a faint characteristic odour and slightly nutty, sweet taste resembling cooked sweet corn.

Identification

Complies with the test of Fatty Acid Composition by Gas Chromatography (2.3.50) and exhibits the following composition profile of fatty acids.

Carbon-Chain length	No. of Double bonds	percentage
<14	0	≤0.1
14	0	≤0.1
16	0	8.6 to 16.5
16	1	≤0.5
18	0	1.0 to 3.3
18	1	20.0 to 42.2
18	2	39.4 to 62.0
18	3	0.5 to 1.5
20	0	≤0.8
20	1	≤0.5
22	0	≤0.3
22	1	≤0.1
24	0	≤0.4

Tests

Weight per ml (2.4.29). 0.914 g to 0.921 g.

Refractive index (2.4.27). 1.470 to 1.474.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Acid value (2.3.23). Not more than 0.2.

Peroxide value (2.3.35). Not more than 10.0.

Iodine value (2.3.28). 102 to 130.

Saponification value (2.3.37). 187 to 193.

Unsaponifiable matter (2.3.39). Not more than 1.5 per cent.

Water (2.3.43). Not more than 0.1 per cent, determined on 0.5 g in a mixture of equal volumes of *decanol* and *methanol*.

Alkaline impurities. Not more than 0.1 ml of *0.01 M hydrochloric acid* is used.

Mix 10 ml of *acetone* and 0.3 ml of *water*, and add 0.05 ml of *bromophenol blue TS*. Neutralize the solution to a green colour if necessary with *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide*. Add 10 ml of Corn Oil, shake, well and allow to stand. Titrate with *0.01 M hydrochloric acid* to change the colour of the upper layer to yellow.

Storage. Store protected from light, moisture and avoid exposure to excessive heat

Labelling. The label states the name and quantity of any added antioxidant.

Cortisone Acetate

$C_{23}H_{30}O_6$ Mol. Wt 402.5

Cortisone Acetate is 17α,21-dihydroxypregn-4-ene-3,11,20-trione 21-acetate.

Cortisone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{23}H_{30}O_6$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cortisone acetate IPRS* or with the reference spectrum of cortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide.*

Mobile phase. Chloroform.

Test solution. Dissolve 25 mg of the substance under examination in 10.0 ml of the same solvent mixture.

Reference solution (a). Dissolve 25 mg of *cortisone acetate IPRS* in 10.0 ml of the same solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v).* Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. 10 mg gives the reactions of acetyl groups (2.3.1).

Tests

Specific optical rotation (2.4.22). +211° to +220°, determined in a 1.0 per cent w/v solution in *dioxan.*

Light absorption (2.4.7). Dissolve 50 mg in sufficient *ethanol (95 per cent)* to produce 100 ml and dilute 2 ml to 100 ml with the same solvent. Absorbance of the resulting solution at the maximum at about 240 nm, 0.375 to 0.405.

Related substances. Determine by liquid chromatography (2.4.14).

Prepare the following solutions immediately before use.

Test solution. Dissolve 25.0 mg of the substance under examination in *acetonitrile* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 2 mg of *cortisone acetate IPRS* and 2 mg of *hydrocortisone acetate IPRS* in *acetonitrile* and dilute to 100.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 400 ml of *acetonitrile* and 550 ml of *water,* allowed to equilibrate sufficient *water* added to produce 1000 ml and mixed,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for 30 minutes.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the

chromatogram is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes. The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2.

Inject reference solution (b) and the test solution. Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.1 g in *ethanol* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol*. Measure the absorbance at the maximum at about 237 nm (2.4.7).

Calculate the content of $C_{23}H_{30}O_6$ taking 395 as the specific absorbance at 237 nm.

Storage. Store protected from light.

Cortisone Injection

Cortisone Acetate Injection

Cortisone Injection is a sterile suspension of a very fine powder of Cortisone Acetate in Sodium Chloride Injection containing suitable dispersing agents.

Cortisone Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cortisone acetate, $C_{23}H_{30}O_6$.

Usual strength. 25 mg per ml.

Description. A white suspension, which settles on standing, but readily disperses on shaking.

Identification

Extract a volume of the injection containing 0.1 g of Cortisone Acetate with 6 ml of *chloroform*, filter and evaporate the *chloroform*. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cortisone acetate IPRS* or with the reference spectrum of cortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. Chloroform.

Test solution. Dissolve a quantity of injection containing 25 mg of cortisone in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). Dissolve 25 mg of *cortisone acetate IPRS* in 10.0 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. 10 mg gives the reactions of acetyle groups (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.2.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix a quantity of the suspension containing 25 mg of Cortisone Acetate with 15 ml of *isopropyl alcohol*, evaporate to dryness on a steam bath. To the residue add 10 ml of the mobile phase, shake, mix with the aid of ultrasound and filter (such as Whatman GF/C filter).

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v, each of, *cortisone acetate IPRS* and *hydrocortisone acetate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of 400 ml *of acetonitrile* and 550 ml of *water*, allowed to equilibrate and sufficient *water* added to produce 1000 ml,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2.

Inject reference solution (a) and the test solution. Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all the secondary peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Add 50 ml of *methanol* to a quantity of the injection containing about 10 mg of Cortisone Acetate, shake, mix with the aid of ultrasound for 2 minutes, dilute to 100.0 ml with *water*, shake, centrifuge and use the supernatant liquid.

Reference solution. Dilute 50 ml of a solution in *methanol* containing 0.02 per cent w/v, each of, *cortisone acetate IPRS* and *prednisolone IPRS* to 100.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: *methanol (60 per cent)*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to cortisone acetate and prednisolone in the chromatogram obtained is at least 5.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{30}O_6$ in the injection.

Storage. Store protected from light in single dose or multiple dose containers at a temperature not exceeding 30°. It should not be allowed to freeze.

Labelling. The label states (1) the name(s) of the dispersing agent(s) added; (2) that it is not meant to be given by intravenous injection; (3) that the container should be gently shaken before a dose is withdrawn.

Cortisone Tablets

Cortisone Acetate Tablets

Cortisone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cortisone acetate, $C_{23}H_{30}O_6$.

Usual strengths. 5 mg; 25 mg.

Identification

Extract a quantity of the powdered tablets containing 0.1 g of Cortisone Acetate with 5 ml of *chloroform*, filter and evaporate the *chloroform*. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cortisone acetate IPRS* or with the reference spectrum of cortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *dimethylformamide*.

Mobile phase. *Chloroform*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the same solvent mixture.

Reference solution (a). Dissolve 25 mg of *cortisone acetate IPRS* in 10.0 ml of the same solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray

the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. 10 mg gives the reactions of acetyl groups (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (paddle),
Medium. 900 ml of a mixture of a 0.3 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary with the medium, at the maximum at about 242 nm (2.4.7). Calculate the content of $C_{23}H_{30}O_6$ in the medium from the absorbance obtained from a solution of known concentration of *cortisone acetate IPRS* in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{23}H_{30}O_6$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix a quantity of the powdered tablets containing 25 mg of Cortisone Acetate with 10.0 ml of the mobile phase, place in an ultrasonic bath for 10 minutes and filter (such as Whatman GF/C filter).

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v, each of, *cortisone acetate IPRS* and *hydrocortisone acetate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of 400 ml *of acetonitrile* and 550 ml of *water*, allowed to equilibrate and sufficient *water* added to produce 1000 ml,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate, about 12 minutes. The test is not valid unless the resolution between the peaks due to hydrocortisone acetate and cortisone acetate is at least 4.2.

Inject reference solution (a) and the test solution. Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all the secondary peaks is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Powder one tablet, add 50 ml of *ethanol*, shake for 30 minutes and add sufficient *ethanol* to produce 100.0 ml. Centrifuge and dilute a suitable volume of the supernatant liquid containing 0.5 mg of Cortisone Acetate to 50.0 ml with *ethanol*. Measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{23}H_{30}O_6$ taking 390 as the specific absorbance at 240 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Add 50 ml of *methanol* to a quantity of the powder containing about 10 mg of Cortisone Acetate, shake, mix with the aid of ultrasound for 2 minutes, dilute to 100.0ml with *water*, shake, centrifuge and use the supernatant liquid.

Reference solution. Dilute 50 ml of a solution in *methanol* containing 0.02 per cent w/v, each of, *cortisone acetate IPRS* and prednisolone to 100.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: *methanol (60 per cent)*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to cortisone acetate and prednisolone in the chromatogram obtained is at least 5.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{30}O_6$ in the tablets.

Storage. Store protected from light.

Absorbent Cotton

Absorbent Cotton Wool

Absorbent Cotton consists of the new fibres or good quality new combers obtained from the seed coat of various species of the genus Gossypium Linn., cleaned, purified, bleached and carded. It does not contain any compensatory colouring matter.

Category. Surgical dressing.

Description. White, well-carded fibres of average staple length not less than 10 mm, containing not more than traces of leaf residue, seed coat and other impurities. It offers appreciable resistance when pulled and does not shed a significant quantity of dust when shaken gently.

Identification

A. When examined under a microscope, each fibre is seen to consist of a single cell, up to about 4 cm long and up to 40 µm wide, in the form of a flattened tube with thick and rounded walls and often twisted.

B. Treat with *iodinated zinc chloride solution;* the fibres become violet.

C. To 0.1 g add 10 ml of *zinc chloride solution,* heat to 40° and allowed to stand for 2 ½ hours, shaking occasionally; the fibres do not dissolve.

Tests

Solution A. To 15.0 g add 150 ml of *water,* macerate for 2 hours in a closed vessel, decant the liquid, carefully squeezing out the residual liquid with a glass rod and mix. Reserve 10 ml for the test for surface-active substances and filter the remainder.

Acidity or alkalinity.To 25 ml of solution A add 0.1 ml of *dilute phenolphthalein solution;* to another 25 ml add 0.05 ml of *methyl orange solution.* Neither solution shows a pink colour.

Surface-active substances. Into a 25-ml graduated, ground-glass stoppered cylinder with external diameter of 18 to 22 mm, previously rinsed with *sulphuric acid* and then with *water,* add the 10 ml portion of solution A, shake vigorously 30 times in 10 seconds, allow to stand for 1 minute and shake again 30 times. After 5 minutes, any foam present must not cover the entire surface of the liquid.

Absorbency

Apparatus. A dry, cylindrical wire basket, 80 mm high and 50 mm in diameter, fabricated from wire of diameter 0.4 mm and having a mesh aperture of 15 to 20 mm; the basket weighs 2.4 to 3.0 g.

Sinking time. Not more than 10 seconds, determined by the following method.

Weigh the basket to the nearest 10 mg. Take five samples, each of approximately 1 g, from different places in the material being examined, pack loosely in the basket and weigh the packed basket to the nearest 10 mg. Hold the basket with its long axis in the horizontal position and drop it from a height of about 10 mm into *water* at 25° contained in a beaker at least 12 cm in diameter and filled to a depth of 10 cm. Measure with a stopwatch the time taken by the basket to sink below the surface of the water. Repeat the procedure on two further samples and calculate the average value.

Water-holding capacity. Not less than 23.0 g per g, determined by the following method.

After the sinking time has been recorded in test A, remove the basket from the water, allow it to drain for exactly 30 seconds with its long axis in the horizontal position, transfer it to a tared beaker and weigh to the nearest 10 mg. Calculate the weight of water retained by the sample. Repeat the procedure on two further samples and calculate the average value.

Foreign fibres. When examined under a microscope, it is seen to consist exclusively of typical cotton fibres, except that occasionally a few isolated foreign fibres may be seen.

Fluorescence. Examine a layer about 5 mm in thickness under ultraviolet light at 365 nm. It shows only a slight, brownish-violet fluorescence and a few yellow particles. Not more than a few isolated fibres show an intense blue fluorescence.

Colouring matter. Slowly extract 10 g in a narrow percolator with *ethanol (95 per cent)* until 50 ml of extract is obtained. The extract is not more intensely coloured than reference solution YS5 or GYS6 (2.4.1) or a solution prepared in the following manner. To 3.0 ml of CSS add 7.0 ml of a solution of *hydrochloric acid* containing 1.0 per cent v/v of *hydrochloric acid* and dilute 0.5 ml of the resulting solution to 10 ml with the same solution of *hydrochloric acid*.

Ether-soluble substances. Not more than 0.5 per cent determined by the following method. Extract 5 g with *ether* in a continuous extraction apparatus (2.1.8), for 4 hours in such a way that the rate is at least four extractions per hour. Evaporate the ether and dry the residue to constant weight at 105°.

Water-soluble substances. Not more than 0.5 per cent, determined by the following method. Boil 5 g with 500 ml of *water* for 30 minutes, stirring frequently and replacing the water lost by evaporation. Decant the liquid into a beaker, squeeze the residual liquid from the material carefully with a glass rod, mix the liquids and filter the extract. Evaporate 400 ml of the filtrate (corresponding to 4/5 of the mass of the sample taken) and dry the residue to constant weight at 105°.

Neps. A thin layer approximately equivalent to 0.5 g for an area of 450 sq. cm. spread uniformly between two glass plates, and viewed by the naked eye under transmitted light, does not show more neps than about an average of 250 for three tests.

Sulphated ash (2.3.18). Not more than 0.5 per cent, determined on 5.0 g, using 2 ml of *sulphuric acid*.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 5.0 g by drying in an oven at 105°.

Storage. Store in a dust-proof package, in a dry place.

Cottonseed Oil

Cottonseed Oil is the refined fixed oil obtained from the seed of cultivated plants of various varieties of *Gossypium hirsutum* Linne or of other species of *Gossypium* (Fam. Malvaceae). It may contain suitable antioxidants.

Category. Pharmaceutical aid.

Description. A Pale yellow or bright golden yellow-coloured, clear oily liquid. It is odourless, or nearly so, with a bland, nutty taste. At temperatures below 10° particles of solid fat may separate from the oil, and at about -5° to 0° the oil becomes solid or nearly so. If it solidifies, the oil should be remelted and thoroughly mixed before use.

Identification

Complies with the test of Fatty Acid Composition by Gas chromatography, Method A (2.3.50) and exhibits the following composition profile of fatty acids.

Carbon-Chain length	No. of Double bonds	percentage
<14	0 or 1	≤0.2
14	0	0.3 to 1.0
16	0	18.0 to 26.4
16	1	≤1.2
18	0	2.1 to 3.3
18	1	14.0 to 21.7
18	2	46.7 to 58.3
18	3	≤1.0
20	0	≤1.0
20	1	≤0.5
22	0	≤0.6
22	1	≤0.5
24	0	≤0.5

Tests

Weight per ml (2.4.29). 0.915 g to 0.921 g.

Refractive index (2.4.27). 1.4645 to 1.4655.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Acid value (2.3.23). Not more than 0.2.

Peroxide value (2.3.35). Not more than 10.0.

Iodine value (2.3.28). 109 to 120.

Unsaponifiable matter (2.3.39). Not more than 1.5 per cent.

Water (2.3.43). Not more than 0.1 per cent, determined on 0.5 g.

Alkaline impurities. Not more than 0.1 ml of *0.01 M hydrochloric acid* is used.

Mix 10 ml of *acetone* and 0.3 ml of *water*, and add 0.05 ml of *bromophenol blue*. Neutralize the solution to a green colour, if necessary, with *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide*. Add 10 ml of cotton seed oil, shake, and allow to stand. Titrate with *0.01 M hydrochloric acid* to change the color of the upper layer to yellow.

Storage. Store protected from light, moisture and avoid exposure to excessive heat.

Labelling. The label states the name and quantity of any added antioxidant.

Cresol

Cresol is a mixture of cresols and other phenols obtained from coal tar.

Category. Disinfectant; pharmaceutical aid (antimicrobial preservative).

Description. An almost colourless to pale brownish-yellow liquid, becoming darker on keeping or on exposure to light; odour, resembling that of phenol but more tarry.

Identification

To 0.5 ml add 300 ml of *water*, shake and filter. Divide the filtrate into two parts. To one part add *ferric chloride test solution*; a transient bluish colour is produced. To the other part add *bromine solution*; a pale yellow flocculent precipitate is produced.

Tests

Distillation range (2.4.8). Not more than 2.0 per cent v/v distils below 188° and not less than 80.0 per cent v/v between 195° and 205°.

Acidity. A 2 per cent w/v solution is neutral to *bromocresol purple solution*.

Weight per ml (2.4.29). 1.029 g to 1.044 g.

Hydrocarbons and volatile bases. Place 50 ml in a 500-ml round-bottomed flask, add about 83 ml of a 27 per cent w/v solution of *sodium hydroxide* and 100 ml of *water* and mix thoroughly.

Connect the flask to a splash-bulb and air condenser about 60 cm long, with the end of the air-condenser fitting closely into the neck of a 250-ml pear-shaped separator and passing well into the separator, which has a cylindrical graduated portion above the stopcock. Fill the graduated portion of the separator with *water*. Distil rapidly until 75 ml of distillate has been collected, cooling the separator in running *water*, if necessary. Allow the separator to stand in a vertical position until separation is complete and draw off the aqueous liquid into a titration flask.

Hydrocarbons — Allow the separator to stand for a short time, measure the volume of hydrocarbon oil in the graduated portion and warm if necessary in order to keep the oil in the liquid state; subtract the volume of volatile bases in the hydrocarbon oil, as determined in the following test; not more than 0.5 per cent v/v of hydrocarbon oil is present.

Volatile bases — To the aqueous portion of the distillate obtained in the preceding test, add any aqueous liquid still remaining in the separator and neutralise it if necessary with *0.1 M hydrochloric acid*, using *phenolphthalein solution* as indicator. Titrate with *1 M hydrochloric acid* using *methyl orange solution* as indicator. Wash the oil from the separator into the titration flask with water and again titrate with *1 M hydrochloric acid*. From the volume of additional *1 M hydrochloric acid* calculate the proportion of volatile bases in the hydrocarbon oil. From the total volume of *1 M hydrochloric acid* used in both titrations calculate the proportion of volatile bases in cresol.

1 ml of *1 M hydrochloric acid* is equivalent to 0.08 ml of volatile bases; not more than 0.15 per cent v/v of volatile bases, calculated as pyridine, are present.

Sulphur compounds. Place about 20 ml in a small conical flask. Moisten a piece of filter paper with a 10 per cent w/v solution of *lead acetate* and fix it on the mouth of the flask; heat the flask on a water-bath for 5 minutes; the filter paper shows not more than a light yellow colour.

Non-volatile matter. Not more than 0.1 per cent w/v when evaporated on a water-bath and dried to constant weight at 105°.

Storage. Store protected from light.

Cresol with Soap Solution

Cresol with Soap Solution is prepared by the saponification of a mixture of Cresol with vegetable oils such as cotton seed, linseed, soyabean or similar oils but excluding coconut and palm kernel oils. Alternatively, the mixed fatty acids derived from these oils may be used.

Cresol with Soap Solution contains not less than 47.0 per cent v/v and not more than 53.0 per cent v/v of Cresol.

Category. Disinfectant.

Description. An amber-coloured to reddish-brown liquid; odour, that of cresol; soapy to touch.

Tests

Appearance of solution. 5 ml mixed with 95 ml of *water* forms a clear solution without producing any opalescence on standing for 3 hours.

Alkalinity. Dilute 5 ml with 50 ml of *ethanol (95 per cent)* neutralised to phenol red solution and titrate with *1 M sulphuric acid*, using *phenol red solution* as indicator; not more than 0.6 ml is required.

Hydrocarbons and volatile bases. Distil 120 ml until all the water and 50 ml of cresol have been collected. Place the cresol thus recovered in a 500-ml round-bottomed flask, add about 83 ml of a 27 per cent w/v solution of *sodium hydroxide* and 100 ml of *water* and mix thoroughly. Connect the flask to a splash-bulb and air condenser about 60 cm long, with the end of the air-condenser fitting closely into the neck of a 250-ml pear-shaped separator and passing well into the separator, which has a cylindrical graduated portion above the stopcock. Fill the graduated portion of the separator with *water*. Distil rapidly until 75 ml of distillate has been collected, cooling the separator in running *water*, if necessary. Allow the separator to stand in a vertical position until separation is complete and draw off the aqueous liquid into a titration flask.

Hydrocarbons — Allow the separator to stand for a short time, measure the volume of hydrocarbon oil in the graduated portion and warm if necessary in order to keep the oil in the liquid state; subtract the volume of volatile bases in the hydrocarbon oil, as determined in the following test; not more than 0.5 per cent v/v of hydrocarbon oil is present.

Volatile bases —To the aqueous portion of the distillate obtained in the preceding test, add any aqueous liquid still remaining in the separator and neutralise it if necessary with *0.1 M hydrochloric acid*, using *phenolphthalein solution* as indicator. Titrate with *1 M hydrochloric acid* using *methyl orange solution* as indicator. Wash the oil from the separator into the titration flask with water and again titrate with *1 M hydrochloric acid*. From the volume of additional *1 M hydrochloric acid* calculate the proportion of volatile bases in the hydrocarbon oil. From the total volume of *1 M hydrochloric acid* used in both titrations calculate the proportion of volatile bases in cresol.

1 ml of *1 M hydrochloric acid* is equivalent to 0.08 ml of volatile bases; not more than 0.15 per cent v/v of volatile bases, calculated as pyridine, are present.

Sulphur compounds. Complies with the test for Sulphur compounds described under Cresol.

Assay. To 50 ml, accurately measured, add 150 ml of *kerosene*, mix and add little powdered *pumice stone* and 3 g of *sodium bicarbonate*. Distil into a separator, the rate of distillation being not more than 2 drops per second until the kerosene and cresol have completely distilled. This is indicated by the distillate being yellow in colour. Stop the distillation, add 50 ml of *kerosene* and collect a further 50 ml of the distillate. Discard the lower aqueous layer in the separator, dry the remainder with *anhydrous calcium chloride* and shake with 10 ml of *sulphuric acid (50 per cent w/w)*. Set aside for 2 hours, reject the acid layer and to the kerosene layer add 40 ml of *sodium hydroxide solution* and shake for 5 minutes. Transfer the alkaline layer to a 100-ml volumetric flask and extract the kerosene layer with 20 ml of *sodium hydroxide solution* adding the alkaline layer to that in the 100-ml volumetric flask. Add *sodium hydroxide solution* from a burette to make the volume in the flask to 100 ml. The difference between the burette reading and 40.5 is equal to the volume of cresol in 50 ml of the sample.

Storage. Store protected from light.

Croscarmellose Sodium

Croscarmellose sodium (cross-linked sodium carboxymethyl cellulose) is the sodium salt of a cross-linked, partly *O*-carboxymethylated cellulose.

Category. Excipient.

Description. A white or greyish-white powder.

Identification

A. Shake 1 g with 100 ml of 0.0004 per cent w/v solution of *methylene blue* and allow to settle. The substance under examination absorbs the methylene blue and settles as a blue, fibrous mass.

B. Shake 1 g with 50 ml of *water*. Transfer 1 ml of the mixture to a test-tube, add 1 ml of *water* and 0.05 ml of a freshly prepared 4.0 per cent w/v solution of *α-naphthol* in *methanol*. Incline the test-tube and add carefully 2 ml of *sulphuric acid* down the side so that it forms a lower layer. A reddish-violet colour develops at the interface.

C. The solution prepared from the sulphated ash in the test for Heavy metals (see Tests) gives reaction (a) of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.0, determined on 1.0 per cent w/v solution in *carbon dioxide-free water*.

Degree of substitution. Take 1.0 g in 500 ml conical flask, add 300 ml of a 10 per cent w/v solution of *sodium chloride*, 25.0 ml of *0.1 M sodium hydroxide*, stopper the flask and allow to stand for 5 minutes, shaking occasionally. Add 0.05 ml of *m-cresol purple solution* and about 15 ml of *0.1 M hydrochloric acid* from a burette. Insert the stopper and shake. If the solution is violet, add *0.1 M hydrochloric acid* in 1 ml portions until the solution becomes yellow, shaking after each addition. Titrate with *0.1 M sodium hydroxide* until the colour turns to violet.

Calculate the number of milliequivalents (M) of base required for the neutralisation equivalent to 1 g of dried substance.

Calculate the degree of acid carboxymethyl substitution (A) from the expression:

$$\frac{1150\,M}{(7102 - 412\,M - 80\,C)}$$

where, C = sulphated ash as a percentage

Calculate the degree of sodium carboxymethyl substitution (S) from the expression:

$$\frac{(162 + 58\,A)\,C}{(7102 - 80\,C)}$$

The degree of substitution is the sum of $A + S$ and it is between 0.60 and 0.85, calculated on the dried basis.

Sodium chloride and sodium glycollate. The sum of the percentage contents of sodium chloride and sodium glycollate is not more than 0.5 per cent, calculated on the dried basis.

Sodium chloride. Place 5.0 g in a 250 ml conical flask, add 50 ml of *water* and 5 ml of *strong hydrogen peroxide solution* and heat on a water-bath for 20 minutes stirring occasionally to ensure total hydration. Cool, add 100 ml of *water* and 10 ml of *nitric acid*. Titrate with *0.05 M silver nitrate* determining the end-point potentiometrically (2.4.25) using a silver indicator electrode and a double-junction reference electrode containing a 10 per cent w/v solution of *potassium nitrate* in the outer jacket and a standard filling solution in the inner jacket, and stirring constantly.

1 ml of *0.05 M silver nitrate* is equivalent to 0.002922 g of NaCl.

Sodium glycollate. Place 0.5 g of the substance under examination in a 100 ml beaker. Add 5 ml of *glacial acetic acid* and 5 ml of *water* and stir to ensure total hydration (about 15 minutes). Add 50 ml of *acetone* and 1 g of *sodium chloride*. Stir for several minutes to ensure complete precipitation of the carboxymethylcellulose. Filter through a fast filter paper impregnated with *acetone* into a volumetric flask, rinse the beaker and filter with 30 ml of *acetone* and dilute the filtrate to

100.0 ml with the same solvent. Allow to stand for 24 hours without shaking. Use the clear supernatant to prepare the test solution.

Reference solution. Dissolve 0.1 g of *glycollic acid* in 100 ml of *water*. Use the solution within 30 days. Transfer 1.0 ml, 2.0 ml, 3.0 ml and 4.0 ml of the solution to separate volumetric flasks; dilute the contents of each flask to 5.0 ml with *water*, add 5 ml of *glacial acetic acid*, dilute to 100.0 ml with *acetone* and mix.

Transfer 2.0 ml of the test solution and 2.0 ml of each of the reference solutions to separate 25 ml volumetric flasks. Heat the uncovered flasks for 20 minutes on a water-bath to eliminate *acetone*. Allow to cool and add 5.0 ml of *2,7-dihydroxynaphthalene solution* to each flask. Mix, add a further 15.0 ml of *2,7-dihydroxynaphthalene solution* and mix again. Close the flasks with aluminium foil and heat on a water-bath for 20 minutes. Cool and dilute to 25.0 ml with *sulphuric acid*.

Measure the absorbance (2.4.7) of each solution at 540 nm. Prepare a blank using 2.0 ml of a solution containing 5 per cent v/v each of *glacial acetic acid* and *water* in *acetone*. Prepare a standard curve using the absorbances obtained with the reference solutions. From the standard curve and the absorbance of the test solution, determine the mass, in milligrams, of glycollic acid in the substance under examination, and calculate the content of sodium glycollate from the expression:

$$\frac{10 \times 1.29 \times a}{(100 - b)\,m}$$

where, 1.29 = the factor converting glycollic acid to sodium glycollate
b = loss on drying as a percentage
m = mass of the substance under examination, in grams

Water-soluble substances. Not more than 10.0 per cent. Disperse 10.0 g in 800.0 ml of *water* and stir for 1 minute every 10 minutes during the first 30 minutes. Allow to stand for 1 hour and centrifuge, if necessary. Decant 200.0 ml of the supernatant liquid on to a fast filter paper in a vacuum filtration funnel, apply vacuum and collect 150.0 ml of the filtrate. Evaporate to dryness and dry the residue at 100° to 105° for 4 hours.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Settling volume. 10.0 to 30.0 ml. Place 75 ml of *water* in a 100 ml graduated cylinder and add 1.5 g of the substance under examination in 0.5 g portions, shaking vigorously after each addition. Dilute to 100.0 ml with *water* and shake again until the substance is homogeneously distributed. Allow to stand for 4 hours. Note the volume of the settled mass.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 10^3 CFU per g and total fungal count is not more than 10^2 CFU per g determined by plate count. 1 g is free from *Escherichia coli*.

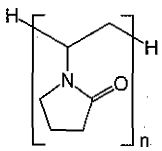
Sulphated ash (2.3.18). 14.0 to 28.0 per cent, calculated on the dried basis.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105° for 6 hours.

Storage. Store protected from moisture.

Crospovidone

1-Ethenyl-2-pyrrolidinone homopolymer; 1-Vinyl-2-pyrrolidinone homopolymer



$(C_6H_9NO)_n$ Mol. Wt. (111.1)$_n$

Crospovidone is a water-insoluble synthetic crosslinked homopolymer of *N*-vinyl-2-pyrrolidinone.

Crospovidone contains not less than 11.0 per cent and not more than 12.8 per cent of nitrogen (N), calculated on the anhydrous basis.

Category. Excipient.

Description. A white to creamy white hygroscopic powder having a faint odour.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6) on specimen previously dried in vacuum at 105° for 1 hour. Compare the spectrum with that obtained with *crospovidone IPRS* or with the reference spectrum of crospovidone.

B. Suspend 1 g in 10 ml of *water*, add 0.1 ml of *0.1 M iodine*, and shake for 30 seconds. Add 1 ml of *starch solution*, and shake; no blue color develops.

Tests

pH (2.4.24). 5.0 to 8.0, determined in a 1.0 per cent w/v aqueous suspension.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Water-soluble substances. Transfer 25.0 g to a 400 ml beaker, add 200 ml of *water*, and stir on a magnetic stirrer, using a 5-cm stirring bar, for 1 hour. Transfer to a 250 ml volumetric flask with the aid of about 25 ml of *water*, add *water* to volume, and mix. Allow the bulk of the solids to settle. Pass about 100 ml of the relatively clear supernatant through a membrane filter having a 0.45 mm porosity, protected against clogging by superimposing a membrane filter. Transfer 50.0 ml of the clear filtrate to a tared 100 ml beaker, evaporate to dryness, and dry at 110° for 3 hours: the weight of the residue does not exceed 75 mg (1.5 per cent).

Impurity A. Determine by liquid chromatography (2.4.14).

Test solution. Shake 1.25 g of the substance under examination in 50.0 ml of *methanol* for 60 minutes and filter.

Reference solution (a). Dissolve 50 mg of *1-vinylpyrrolidin-2-one* in 100.0 ml of the *methanol*. Dilute 1.0 ml of the solution to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *1-vinylpyrrolidin-2-one (crospovidone impurity A IPRS)* and 50 mg of *vinyl acetate* in 10.0 ml of the *methanol*. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of *water*,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 50 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to crospovidone impurity A and vinyl acetate is not less than 2.0.

Inject reference solution (a) and the test solution. The relative standard deviation for replicate injections is not more than 2.0. In the chromatogram obtained with the test solution, the area of secondary peak due to crospovidone impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (10 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

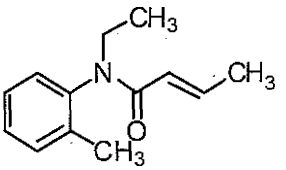
Nitrogen (2.3.30). Place 0.1 g of the substance under examination (*m* mg) in a combustion flask, add 5 g of a mixture of 1 g of *copper sulphate*, 1 g of *titanium dioxide* and 33 g of *dipotassium sulphate*, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of *water*. Add 7 ml of *sulphuric acid*, allowing it to run down the sides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of *sulphuric acid*. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulphuric acid in the neck of the flask; precautions are to be taken to prevent the upper part of the flask from becoming overheated. Continue the heating for 45 minutes. Cool, dissolve the solid material by cautiously adding to the mixture 20 ml of *water*, cool again and place in a steam-distillation apparatus. Add 30 ml of *strong sodium hydroxide solution* through the funnel, rinse the funnel cautiously with 10 ml of *water* and distil immediately by passing steam through the mixture. Collect 80-100 ml of distillate in a mixture of 30 ml of a 4.0 per cent w/v solution of *boric acid* and 0.05 ml of *bromocresol green-methyl red solution* and enough *water* to cover the tip of the condenser. Towards the end of the distillation lower the receiver so that the tip of the condenser is above the surface of the acid solution and rinse the end part of the condenser with a small quantity of *water*. Titrate the distillate with *0.025 M sulphuric acid* until the colour of the solution changes from green through pale greyish-blue to pale greyish-red-purple (n_1 ml of *0.025 M sulphuric acid*).

Repeat the test using about 100 mg of *glucose* in place of the substance under examination (n_2 ml of *0.025 M sulphuric acid*).

$$\text{Percent content of nitrogen} = \frac{0.7004(n_1 - n_2)}{m} \times 100$$

Storage. Store protected from moisture.

Crotamiton



$C_{13}H_{17}NO$ Mol. Wt. 203.3

Crotamiton is (*E*)-*N*-Ethyl-*N*-(2-methylphenyl)-2-butenamide.

Crotamiton is the sum of the (E)- and (Z)-isomers contains not less than 96.0 per cent and not more than 102.0 per cent, and (Z)-isomer is not more than 15.0 per cent.

Category. Scabicide and pediculicide.

Description. A colourless or pale yellow, oily liquid.

Identification

Test B may be omitted if tests A, C and D are carried out. Tests A, C and D may be omitted if test B is carried out.

A. When examined in the range 220 nm to 300 nm (2.4.7), a 0.0025 per cent w/v solution in *cyclohexane* shows an absorption maximum at 242 nm and specific absorbance at the absorption maxima is 300 to 330.

B. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *crotamiton IPRS* or with the reference spectrum of crotamiton.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 97 volumes of the solution prepared by mixing 98.0 ml of *dichloromethane* with 2.0 ml of *ammonia*, dry over *anhydrous sodium sulphate* and 3 volumes of *2-propanol*.

Test solution. Dissolve 25 mg of the substance under examination in *ethanol* and dilute to 10 ml with *ethanol*.

Reference solution. A 0.25 per cent w/v solution of *crotamiton IPRS* in *ethanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. To 10 ml of a saturated solution add a few drops of a 0.3 per cent w/v solution of *potassium permanganate*. A brown colour is obtain and a brown precipitate is formed on standing.

Tests

Relative density (2.4.29). 1.006 to 1.011 at 20°.

Refractive index (2.4.27). 1.540 to 1.542.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Test solution (b). Dilute 1.0 ml of test solution (a) to 20.0 ml with the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *crotamiton IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b). A 0.075 per cent w/v solution of *crotamiton impurity A IPRS* (*N-ethyl-N-(2-methylphenyl)but-3-enamide IPRS)* in the mobile phase. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (d). A 0.015 per cent w/v solution of *crotamiton impurity A IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with test solution (a).

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with silica (5 µm),
- mobile phase: a mixture of 8 volumes of *tetrahydrofuran* and 92 volumes of *cyclohexane*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume: 20 µl.

The relative retention time with reference to *(E)*-isomer for *(Z)*-isomer is about 0.5 and for crotamiton impurity A is about 0.8.

Injection reference solution (d). The test is not valid unless the resolution between the peaks corresponding to crotamiton impurity A and the *(E)*-isomer is not less than 4.5.

Injection reference solution (b), (c) and test solution (a). Run the chromatograms for 2.5 times the retention time of the *(E)*-isomer. In the chromatogram obtained with test solution (a) the area of any peak corresponding to crotamiton impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (3.0 per cent), the area of any other secondary peak is not more than 0.1 times the sum of the areas of the peaks corresponding to the *(Z)*- and *(E)*- isomers in the chromatogram obtained with reference solution (c) (0.1 per cent), The sum of areas of all the secondary peaks other than crotamiton impurity A is not more than the sum of the areas of the peaks corresponding to the *(Z)*- and *(E)*-isomers in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peak with an area less than 0.02 times the sum of the areas of the peaks corresponding to the *(Z)*- and *(E)*-isomers in the chromatogram obtained with reference solution (c) (0.02 per cent).

Free amines. Dissolve 5.0 g in 16 ml of *dichloromethane* and add 4.0 ml of *glacial acetic acid*. Add 0.1 ml of *metanil yellow solution* and 1.0 ml of *0.02 M perchloric acid*. The solution is red-violet.

Chlorides. Boil 5.0 g under a reflux condenser for 1 hour with 25 ml of *ethanol (95 per cent)* and 5 ml of a 20.0 per cent w/v solution of *sodium hydroxide*. Cool, add 5 ml of *water* and shake with 25 ml of *ether*. Dilute the lower layer to 20 ml with *water*, add 5 ml of *nitric acid*, dilute to 50 ml with *water* and add 1 ml of a freshly prepared 5.0 per cent w/v solution of *silver nitrate*. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of a freshly prepared 5.0 per cent w/v solution of *silver nitrate* and a solution

prepared by diluting 5 ml of a 20.0 per cent w/v solution of *sodium hydroxide* to 20 ml with *water* and adding 1.5 ml of *0.01 M hydrochloric acid*, 5 ml of *nitric acid* and diluting to 50 ml with *water* (100 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances, using the following modification.

Injection reference solution (a) and test solution (b).

Calculate the content of $C_{13}H_{17}NO$ from the sum of the areas of the peaks corresponding to the (*Z*)- and (*E*)-isomers in the chromatograms obtained. Calculate the content of the (*Z*)-isomer, as a percentage of the total content of the (*E*)- and (*Z*)- isomers, from the chromatogram obtained with test solution (b).

Storage. Store protected from light.

Crotamiton Cream

Crotamiton Cream contains Crotamiton in a suitable cream base.

Crotamiton Cream contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of crotamiton, $C_{13}H_{17}NO$.

Usual strength. 10 per cent w/w.

Identification

A. Mix a quantity of the cream containing 0.5 g of Crotamiton with 150 ml of *water* and then slowly add 50 ml of *1 M sodium hydroxide* while stirring vigorously. Filter the mixture, adjusted the filtrate to pH 7 with *5 M hydrochloric acid* and extract with 50 ml of *ether*. Wash the ether layer with 10 ml of a saturated solution of *sodium chloride*, dry the organic layer over *anhydrous sodium sulphate*, filter and evaporate to an oily residue. When examined in the range 220 nm to 350 nm (2.4.7), a 0.003 per cet w/v solution of the residue in *cyclohexane*, shows absorption maximum only at 242 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 97 volumes of the solution prepared by shaking 98 ml of *chloroform* with 2 ml of *18 M ammonia*, dry over *anhydrous sodium sulphate* and 3 volumes of *2-propanol*.

Test solution. A 0.25 per cent w/v of the residue obtained in test A with *ethanol*.

Reference solution (a). A 0.25 per cent w/v of *crotamiton IPRS* in *ethanol*.

Reference solution (b). A mixture of equal volumes of the test solution and reference soluton (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise the plate to 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a), but if not, the principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

C. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the principal peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay, using following modification.

Inject reference solution (b), (c) , (e) and the test solution (a). Run the chromatogram 2.5 the retention time of the principal peak for test solution (a), the area of any peak corresponding to crotamiton impurity A is not more than the area of corresponding peak in the chromatogram obtained with reference solution (b) (3 per cent), the sum of the areas of all the secondary peaks other than the peaks corresponding to the *Z*-isomer and to crotamiton impurity A is not more than the sum of the areas of the peaks corresponding to the *E*- and *Z*-isomers in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.02 per cent) and any peak with the same retention time as the principal peak in the chromatogram obtained with reference solution (e).

***Z*-Isomer**. Not more than 15 per cent of the total content of *E*- and *Z*-isomers determined in the Assay.

Other tests. Comply with the tests stated under Cream.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Add 2 ml of *water* and 100 ml of *cyclohexane* to a quantity of the preparation under examination containing 0.1 g of Crotamiton, shake for 10 minutes and separate the lower, aqueous layer. Repeat the extraction using two 10-ml quantities of *cyclohexane*, filter the combined extracts and add sufficient *cyclohexane* to obtain 200.0 ml.

Test solution (b). Dilute 1.0 ml of test solution (a) to 20.0 ml with *cyclohexane*.

Reference solution (a). A 0.0025 per cent w/v solution of *crotamiton IPRS* in *cyclohexane*.

Reference solution (b). A 0.0015 per cent w/v solution of *crotamiton impurity A IPRS (N-ethyl-N-(2-methylphenyl)but-3-enamide IPRS)* in *cyclohexane*.

Reference solution (c). Dilute 1.0 ml of test solution (a) to 100.0 ml with *cyclohexane.*

Reference solution (d). Dilute 1.0 ml of 0.015 per cent w/v solution of *crotamiton impurity A IPRS* to 10.0 ml with test solution (a).

Reference solution (e). A 0.001 per cent w/v solution of *methyl hydroxybenzoate* in *cyclohexane.*

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with silica gel (5 µm) (Such as Lichrosorb Si60),
- mobile phase: a mixture of 8 volumes of *tetrahydrofuran* and 92 volumes of *cyclohexane,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume: 20 µl.

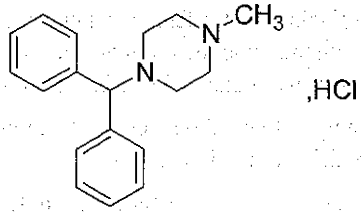
The relative retention time with reference to E-crotamiton for (*Z*)-isomer is about 0.5 and for crotamiton impurity A is about 0.8.

Inject reference solution (d). The test is not valid unless the resolution between the peaks corresponding to the (*E*)-isomer and crotamiton impurity A is not less than 4.5.

Inject reference solution (a), (b) and test solution (b).

Calculate the content of $C_{13}H_{17}NO$ by summing E and Z-isomers.

Cyclizine Hydrochloride



$C_{18}H_{22}N_2$, HCl Mol. Wt. 302.8

Cyclizine Hydrochloride is 1-(diphenylmethyl)-4-methylpiperazine hydrochloride.

Cyclizine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{18}H_{22}N_2$,HCl, calculated on the dried basis.

Category. Antiemetic.

Description. A white, crystalline powder.

Identification

Tests B and C may be omitted if tests A and D are carried out. Test A may be omitted if tests B, C and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclizine hydrochloride IPRS* or with the reference spectrum of cyclizine hydrochloride.

B. When examined in the range 220 nm to 360 nm, a freshly prepared 0.002 per cent w/v solution in *0.05 M sulphuric acid* shows absorption maximum only at about 225 nm, about 0.78 (2.4.7).

C. Dissolve 0.5 g in 10 ml of *ethanol (95 per cent),* warming if necessary, cool in ice, add 1 ml of *5 M sodium hydroxide* and sufficient *water* to produce 20 ml. Stir well and filter; the precipitate, after washing with *water* and drying at 60° at a pressure not exceeding 0.7 kPa for 2 hours, melts at about 107° (2.4.21).

D. Gives the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by gas chromatography (2.4.13).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 0.25 g of the substance under examination in 4.0 ml of *methanol* and dilute to 5.0 ml with *1 M sodium hydroxide.*

Reference solution (a). A 0.005 per cent w/v solution of *cyclizine hydrochloride IPRS* in *methanol.*

Reference solution (b). A solution containing 0.025 per cent w/v each of the substance under examination, *cyclizine impurity A IPRS* and *cyclizine impurity B IPRS* in *methanol.*

Chromatographic system

- a fused silica column 25 m x 0.33 mm, packed with poly(dimethyl)(diphenyl)siloxane (film thickness 0.5 µm),
- temperature:

column	time (min.)	temperature (°)
	0-14	100→240
	14-16	240→270
	16-30	270

- inlet port at 250 and detector at 290°,
- split ratio. 1:25,
- flame ionization detector,
- flow rate: 1 ml per minute using nitrogen as the carrier gas.

Name	Relative retention time
Cyclizine impurity A[1]	0.2
Cyclizine impurity B[2]	0.7
Cyclizine (Retention time: about 15 minutes)	1.0

[1]1-methylpiperazine,

[2]diphenylmethanol.

Inject 1 µl of reference solution (b). The test is not valid unless peak-to-valley ratio is not less than 50, where H_p is the height above the baseline of the peak due to cyclizine impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to methanol.

Inject 1 µl of reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to cyclizine impurities A and B is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 130°.

Assay. Weigh 0.1 g, dissolve in 20 ml of *anhydrous glacial acetic acid* and add 50 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01514 g of $C_{18}H_{22}N_2,HCl$.

Storage. Store protected from light.

Cyclizine Tablets

Cyclizine Hydrochloride Tablets

Cyclizine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cyclizine hydrochloride, $C_{18}H_{22}N_2,HCl$.

Usual strength. 50 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.1 g of Cyclizine Hydrochloride with 10 ml of *ethanol (95 per cent)*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclizine hydrochloride IPRS* or with the reference spectrum of cyclizine hydrochloride.

B. Extract a quantity of the powdered tablets containing 0.5 g of Cyclizine Hydrochloride with 20 ml of *water* and filter. The filtrate gives reaction A of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate suitably diluted with 1 per cent v/v of *sulphuric acid*, at the maximum at about 264 nm. (2.4.7). Calculate the content of $C_{18}H_{22}N_2,HCl$ in the medium from the absorbance obtained from a solution of known concentration of *cyclizine hydrochloride IPRS* in 1 per cent v/v of *sulphuric acid*.

Q. Not less than 75 per cent of the stated amount of $C_{18}H_{22}N_2,HCl$.

***N*-Methylpiperazine.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 8 volumes of *methanol and* 2 volumes of *strong ammonia solution*.

NOTE — Prepare the following solutions freshly.

Test solution. Triturate a quantity of the powdered tablets containing 0.1 g of Cyclizine Hydrochloride with 10 ml of *methanol* and filter.

Reference solution. A 0.005 per cent w/v solution of *N-methylpiperazine IPRS* in *methanol*.

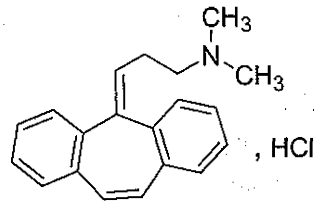
Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose to iodine vapours for 10 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.125 g of Cyclizine Hydrochloride and shake with 400 ml of *0.05 M sulphuric acid* for 15 minutes. Add sufficient *0.05 M sulphuric acid* to produce 500.0 ml and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with *0.05 M sulphuric acid* and measure the absorbance of the resulting solution at the maximum at about 225 nm (2.4.7). Calculate the content of $C_{18}H_{22}N_2,HCl$ taking 390 as the specific absorbance at 225 nm.

Storage. Store protected from light.

Cyclobenzaprine Hydrochloride



$C_{20}H_{21}N,HCl$ Mol Wt. 311.9

Cyclobenzaprine Hydrochloride is1-propanamine,3-(5*H*-dibenzo[a,d]cyclohepten-5-ylidene)-*N*,*N*-dimethyl-hydrochloride; *N*,*N*-Dimethyl-5*H*-dibenzo[a,d]cycloheptene-δ^5,γ-propylamine hydrochloride.

Cyclobenzaprine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{21}N,HCl$ calculated on the dried basis.

Category. Muscle relaxant.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *cyclobenzaprine hydrochloride IPRS* or with the reference spectrum of cyclobenzaprine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

C. It gives a reaction (a) of chloride (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substances under examination in 100.0 ml of the mobile phase.

Reference solution. A solution containing 0.04 per cent w/v solution of *cyclobenzaprine hydrochloride IPRS* and each of 0.00006 per cent *cyclobenzaprine related compound A IPRS* and *cyclobenzaprine related compound B IPRS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octaylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 4.0 g of *ammonium acetate* in 350 ml of *water*. Add 650 ml of *methanol* and adjusted to pH 7.2 with *dilute ammonia solution* or *dilute acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Cyclobenzaprine related compound A	0.51	1.51
Cyclobenzaprine related compound B	0.59	1.0
Cyclobenzaprine N-oxide[1]	0.74	1.08
Cyclobenzaprine	1.0	—
Amitryptyline[2]	1.3	2.78
Dibenzocycloheptenone[3]	1.6	1.56

[1]3-(5*H*-Dibenzo[a,d]cyclohepten-5-ylidene)-*N*,*N*-dimethyl-1-propanamine *N*-oxide,

[2]10,11-Dihydro-*N*,*N*-dimethyl-5*H*-dibenzo[a,d]cycloheptene-δ^5,γ-propylamine,

[3]Dibenzo[a,d]cyclohepten-5-one.

Inject the reference solution. Run the chromatogram 3 times of the principal peak. The test is not valid unless the resolution between the cyclobenzaprine related compound A and cyclobenzaprine related compound B is not less than 2.0. The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution. The area of peak due to cyclobenzaprine related compound A, the area of peak due of cyclobenzaprine related compound B, the area of peak due to cyclobenzaprine N-oxide, the area of peak due to cyclobenzaprine, the area of peak due to amitryptyline and the area of peak due to dibenzocycloheptenone is not more than (0.15 per cent), the area of any other impurity is not more than (0.1 per cent) and sum of area of all the secondary peak is not more than (1.0 per cent), calculated by area normalization.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substances under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *cyclobenzaprine hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *methanol* and 35 volumes of a buffer solution prepared by dissolving 2.0 g of *ammonium acetate* in 1000 ml of *water* and

adjusted to pH 8.9 with 25 per cent w/v solution of *ammonium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 per cent and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}N,HCl$.

Storage. Store protected from moisture.

Cyclobenzaprine Tablets

Cyclobenzaprine Hydrochloride Tablets

Cyclobenzaprine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyclobenzaprine hydrochloride, $C_{20}H_{21}N,HCl$.

Usual strengths. 5 mg, 10 mg.

Identification

A. Dissolve a quantity of the powdered tablet containing 50 mg of cyclobenzaprine hydrochloride from a quantity of finely powdered tablets, to a small flask. Add 10 ml of *methylene chloride*, swirl to dissolve, and filter. Evaporate the clear filtrate to about 5 ml, transfer to a centrifuge tube, and add 1 to 2 ml of *ether*. Evaporate about 1 ml of *ether* and agitate until crystallization occurs. Wash the crystals with several portions of *ether*, and dry in air. On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclobenzaprine hydrochloride IPRS* or with the reference spectrum of cyclobenzaprine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution

Tests

Dissolution (2.5.2).
Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted, if necessary with dissolution medium at 290 nm. Calculate the content of cyclobenzaprine hydrochloride, in the medium from the absorbance obtained from a solution of known concentration of *cyclobenzaprine hydrochloride IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{21}N,HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 40 mg of cyclobenzaprine hydrochloride in 100-ml volumetric flask. Add mobile phase and sonicate for 30 minutes. Allow the solution to cool to room temperature, and then dilute with mobile phase to volume. Centrifuge the solution, and use the supernatant.

Reference solution. A 0.00006 per cent w/v solution of *cyclobenzaprine hydrochloride IPRS*, *cyclobenzaprine related compound A IPRS* and *cyclobenzaprine related compound B IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture 65 volumes of *methanol* and 35 volumes of a buffer solution prepared by dissolving 11.4 g of *ammonium acetate* in 1000 ml of *water* and adjusted to pH 7.2 with *dilute ammonium solution*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 10 µl.

Name	Relative retention time
Cyclobenzaprine related compound A[1]	0.51
Cyclobenzaprine related compound B[1]	0.59
Cyclobenzaprine *N*-oxide[2]	0.74
Cyclobenzaprine	1.0
Amitriptyline[1,3]	1.3
Dibenzocycloheptenone[4]	1.6

[1]Process impurity include for identification only and not included in the calculation of total degradation products,

[2]3-(5*H*-dibenzo[a,d]cyclohepten-5-ylidene)-*N*,*N*-dimethyl-1-propanamine *N*-oxide,

[3]10,11-Dihydro-*N*,*N*,-dimethyl-5*H*-dibenzo[a,d]cycloheptene-propylamine,

[4]Dibenzo[*a*,*d*]cyclohepten-5-one.

Inject the reference solution. Run the chromatogram 3 times of principle peak. The test is not valid unless the resolution between the cyclobenzaprine related compound A and cyclobenzaprine related compound B is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to impurity cyclobenzaprine N-oxide is

not more than the area of corresponding peak obtained with the reference solution (0.15 per cent), in the chromatogram obtained with the test solution the area of any peak corresponding to impurity dibenzocycloheptenone is not more than the area of corresponding peak obtained with the reference solution (0.15 per cent), the area of any other impurity is not more than 0.66 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the area of total impurity is not more than 13.33 times the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

Test solution. To one tablet add 20 ml of the mobile phase, disperse with the aid of ultrasound for 10 minutes, dilute, if necessary, with sufficient of the mobile phase to produce a solution containing 0.02 per cent of cyclobenzaprine hydrochloride and filter.

Calculate the content of $C_{20}H_{21}N,HCl$ in the Tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Cyclobenzaprine Hydrochloride in 100.0 ml volumetric flask. Add mobile phase and sonicate for 30 minutes. Allow the solution to cool to room temperature, and then dilute with mobile phase to volume. Centrifuge the solution, and use the supernatant.

Reference solution. A 0.02 per cent w/v solution of *cyclobenzaprine hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *methanol* and 35 volumes of a buffer solution prepared by dissolving 11.4 g of *ammonium acetate* in 1000 ml of *water* and adjusted to pH 7.2 with *ammonium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 per cent and the relative standard deviation for replicate injections is not more than 0.85 per cent.

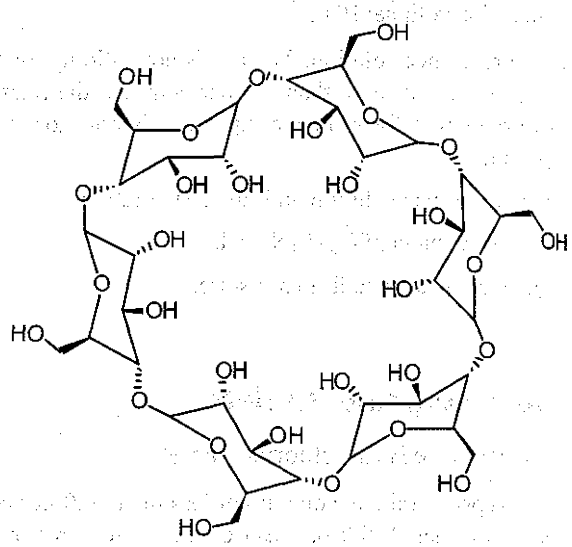
Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}N,HCl$ in tablets.

Storage. Store protected from moisture.

Alfa-Cyclodextrin

α-cyclodextrin; Alfadex



$C_{36}H_{60}O_{30}$ Mol. Wt. 972.8

Alfa-Cyclodextrin is six alpha-(1-4) linked glucopyranosyl units.

Alfa-Cyclodextrin contains not less than 98.0 per cent and not more than 101.0 per cent of $(C_6H_{10}O_5)_6$, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Description. A white, or almost white, amorphous or crystalline powder.

Identification

Tests B and C may be omitted if tests A and D are carried out. Test A may be omitted if tests B, C and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *alfa-cyclodextrin IPRS* or with the reference spectrum of alfa-cyclodextrin.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

C. Mix 0.2 g with 2 ml of *iodine solution*, warm in a water-bath to dissolve and allow to stand at room temperature; a yellow-brown precipitate is formed.

D. Specific optical rotation (see Tests).

Tests

Specific optical rotation (2.4.22). +147° to +152°, determined in 1.0 per cent w/v solution at 20°.

pH (2.4.24). 5.0 to 8.0, determined on a mixture of 30 ml of 1.0 per cent w/v of alfa-cyclodextrin and 1 ml of 22.4 per cent w/v of *potassium chloride*.

Reducing sugars

Test solution. To 1 ml of 1.0 per cent w/v solution, add 1 ml of *cupri-tartaric solution.* Heat on a water-bath for 10 minutes, cool to room temperature. Add 10 ml of *ammonium molybdate reagent* and allow to stand for 15 minutes.

Reference solution. To 1 ml of a 0.002 per cent w/v solution of *glucose*, add 1 ml of *cupri-tartaric solution.* Heat on a water-bath for 10 minutes, cool to room temperature. Add 10 ml of *ammonium molybdate reagent* and allow to stand for 15 minutes.

Measure the absorbance at the maximum at 740 nm (2.4.7) using *water* as blank. The absorbance of the test solution is not more than that of the reference solution (0.2 per cent).

Light-absorbing impurities. When a 1.0 per cent w/v solution examined in the range 230 nm to 350 nm (2.4.7), the absorbance is not more than 0.1 and in the range 350 nm to 750 nm (2.4.7), the absorbance is not more than 0.05.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay with the following modifications.

Inject reference solution (b) and test solution (a). Run the chromatogram 3.5 times the retention time of the principal peak. In the chromatogram obtained with test solution (a), the area of any peak corresponding to betadex or gamma-cyclodextrin is not more than 0.5 times the area of the corresponding peaks in the chromatogram obtained with reference solution (b) (0.25 per cent) and the sum of areas of all the secondary peaks other than the peaks corresponding to betadex or gamma-cyclodextrin, is not more than 0.5 times the area of the peak corresponding to alfadex in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 11.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 0.25 g of the substance under examination in *water* with heating, cool and dilute to 25.0 ml with *water.*

Test solution (b). Dilute 5.0 ml of test solution (a) to 50.0 ml with *water.*

Reference solution (a). A solution containing 0.05 per cent w/v, each of, *beta-cyclodextrin IPRS*, *gamma-cyclodextrin IPRS* and 0.1 per cent w/v of *alfa-cyclodextrin IPRS* in *water.*

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with *water.*

Reference solution (c). A 0.1 per cent w/v solution of *alfa-cyclodextrin IPRS* in *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 10 volumes of *methanol* and 90 volumes of *water*,
- flow rate: 1.5 ml per minute,
- a differential refractometer,
- injection volume: 50 µl.

Equilibrate the column atleast for about 3 hours.

Name	Relative retention time
Gamma-cyclodextrin	0.7
Alfa-cyclodextrin (retention time: about 10 minutes)	1.0
Beta-cyclodextrin	2.2

Inject reference solution (a). Run the chromatograms for 3.5 times the retention time of the principal peak. The test is not valid unless the resolution between the peaks corresponding to gamma-cyclodextrin and alfadex is not less than 1.5 and the relative standard deviation for replicate injections due to alfa-cyclodextrin is not more than 2.0 per cent.

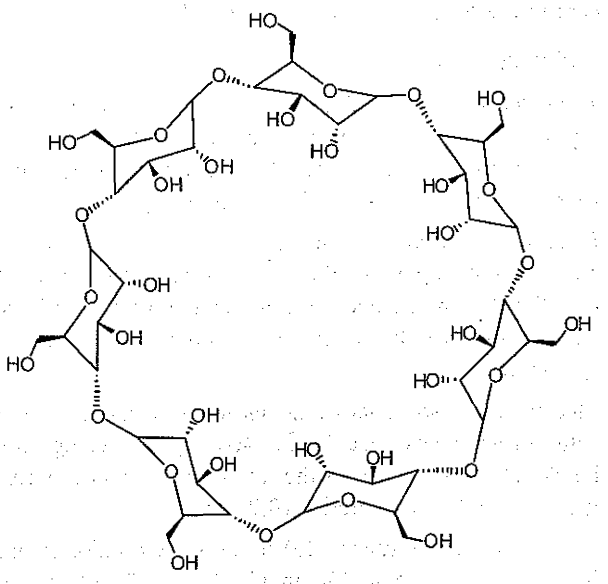
Inject reference solution (c) and test solution (b).

Calculate the content of $(C_6H_{10}O_5)_6$.

Storage. Store protected from moisture.

Beta-Cyclodextrin

β-cyclodextrin; Betadex



$C_{42}H_{70}O_{35}$ Mol. Wt. 1135.0

Beta-cyclodextrin is composed of seven alpha-(1-4) linked D-glucopyranosyl units.

Beta-cyclodextrin contains not less than 98.0 per cent and not more than 101.0 per cent of $(C_6H_{10}O_5)_7$, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Description. A white or almost white, amorphous or crystalline powder.

Identification

Tests B and C may be omitted if tests A and D are carried out. Test A may be omitted if tests B, C and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *beta-cyclodextrin IPRS* or with the reference spectrum of beta-cyclodextrin.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

C. Mix 0.2 g with 2 ml of *iodine solution*, warm in a water-bath to dissolve and allow to stand at room temperature; a yellow-brown precipitate is formed.

D. Specific optical rotation (see Tests).

Tests

Specific optical rotation (2.4.22). +160.0° to +164.0°, determined in a 1.0 per cent w/v solution at 20°.

pH (2.4.24). 5.0 to 8.0, determined in a mixture of 30 ml of 1.0 per cent w/v solution of beta-cyclodextrin and 1 ml of 22.36 per cent w/v solution of *potassium chloride*.

Reducing sugars

Test solution. To 1 ml of 1.0 per cent w/v solution, add 1 ml of *cupri-tartaric solution*. Heat on a water-bath for 10 minutes, cool to room temperature. Add 10 ml of *ammonium molybdate reagent* and allow to stand for 15 minutes.

Reference solution. To 1 ml of a 0.002 per cent w/v solution of *glucose*, add 1 ml of *cupri-tartaric solution*. Heat on a water-bath for 10 minutes, cool to room temperature. Add 10 ml of *ammonium molybdate reagent* and allow to stand for 15 minutes.

Measure the absorbance of the test solution and the reference solution at the maxima at about 740 nm (2.4.7) using *water* as blank. The absorbance of the test solution is not more than that of the reference solution (0.2 per cent).

Light absorption. When a 1.0 per cent w/v solution examined in the range 230 nm to 350 nm (2.4.7), the absorbance is not more than 0.1 and in the range 350 nm to 750 nm, the absorbance is not more than 0.05.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Inject reference solution (b) and test solution (a). Run the chromatogram 1.5 times the retention time of peak due to beta-cyclodextrin. In the chromatogram obtained with test solution (a), the areas of any peaks corresponding to gamma-cyclodextrin and alfa-cyclodextrin are not more than 0.5 times the area of the corresponding peaks in the chromatogram obtained with reference solution (b) (0.25 per cent) and the sum of areas of all other secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 14.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 0.25 g of the substance under examination in *water* with heating, cool and dilute to 25.0 ml with *water*.

Test solution (b). Dilute 5.0 ml of test solution (a) to 50.0 ml with *water*.

Reference solution (a). A solution containing 0.05 per cent w/v, each of *alfa-cyclodextrin IPRS* and *gamma-cyclodextrin IPRS* and 0.1 per cent w/v of *beta-cyclodextrin IPRS* in *water*.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with *water*.

Reference solution (c). A 0.1 per cent w/v solution of *beta-cyclodextrin IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 10 volumes of *methanol* and 90 volumes of *water*,
- flow rate: 1.5 ml per minute,
- a differential refractometer,
- injection volume: 50 µl.

Equilibrate the column for about 3 hours.

Name	Relative retention time
Gamma-cyclodextrin	0.3
Alfa-cyclodextrin	0.45
Beta-cyclodextrin	1.0

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to gammacyclodextrin and

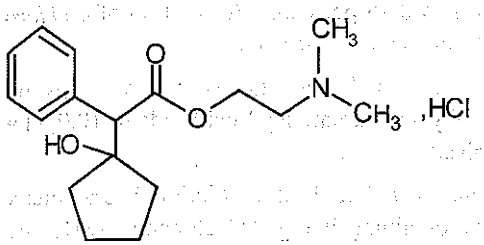
alfadex is not less than 1.5 and the relative standard deviation for replicate injections for beta-cyclodextrin is not more than 2.0 per cent.

Inject reference solution (c) and test solution (b).

Calculate the content of $(C_6H_{10}O_5)_7$.

Storage. Store protected from moisture.

Cyclopentolate Hydrochloride



$C_{17}H_{25}NO_3,HCl$ Mol. Wt.327.8

Cyclopentolate Hydrochloride is 2-(dimethylamino)ethyl α-(1-hydroxycyclopentyl)(phenyl)acetate hydrochloride.

Cyclopentolate Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{17}H_{25}NO_3,HCl$, calculated on the dried basis.

Category. Anticholinergic.

Description. A white or almost white, crystalline powder. It shows polymorphism (2.5.11).

Identification

Test B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclopentolate hydrochloride IPRS* or with the reference spectrum of cyclopentolate hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *ammonia*, 15 volumes of *water*, 30 volumes of *butyl acetate* and 50 volumes of *2-propanol*.

Test solution. Dissolve 10 mg of the substance under examination in 5 ml of *ethanol (95 per cent)*.

Reference solution. A 0.2 per cent w/v solution of *cyclopentolate hydrochloride IPRS* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with alcoholic solution of *sulphuric acid* and heat at 120° for 30 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

C. It gives reaction (a) of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 5.5, determined in a 1.0 per cent w/v solution in *carbon dioxide free water*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Prepare the solutions immediately before use.

Test solution. Dissolve 20 mg of the substance under examination in *water* and dilute to 20.0 ml with *water*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with endcapped hexylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of a solution prepared by dissolving 0.66 g of *ammonium phosphate* in *water*, adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water* and 45 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Cyclopentolate impurity C[1]	0.9	2.0
Cyclopentolate (Retention time: about 4 minutes)	1.0	—

[1]2-(dimethylamino) ethyl phenylacetate.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to cyclopentolate impurity C is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal

peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Dissolve 0.25 g in a mixture of 1.0 ml of *0.1 M hydrochloric acid* and 50 ml of *ethanol (95 per cent)*. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (2.4.25). Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03279 g of $C_{17}H_{26}ClNO_3$.

Cyclopentolate Eye Drops

Cyclopentolate Hydrochloride Eye Drops

Cyclopentolate Eye Drops are a sterile solution of Cyclopentolate Hydrochloride in Purified Water.

Cyclopentolate Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyclopentolate hydrochloride $C_{17}H_{25}NO_3$,HCl.

Usual strength. 1 per cent w/v.

Identification

Add *2 M ammonia* to a volume of the eye drops containing 25 mg of Cyclopentolate Hydrochloride until alkaline and extract immediately with 50 ml of *ether*. Wash the extract with 5 ml of *water*, filter through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. On the oily residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclopentolate hydrochloride IPRS* treated in the same manner or with the reference spectrum of cyclopentolate.

Tests

pH (2.4.24). 3.0 to 5.5.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *13.5 M ammonia*, 15 volumes of *water*, 30 volumes of *butyl acetate* and 50 volumes of *propan-2-ol*.

Test solution. Dilute a volume of eye drops to obtain a solution containing 0.5 per cent w/v of Cyclopentolate Hydrochloride in *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with *water*.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with *water*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 120° for 5 minutes, spray with *ethanolic sulphuric acid (10 per cent)*, heat at 120° for 30 minutes and examine under ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Solution A. A 0.25 per cent w/v solution of *4-chlorophenol* (internal standard) in *methanol*.

Test solution (a). Dilute a volume of the eye drops containing 20 mg of Cyclopentolate Hydrochloride to 10.0 ml with the mobile phase.

Test solution (b). Add 4 ml of solution A to a volume of the eye drops containing 20 mg of Cyclopentolate Hydrochloride and dilute to 10.0 ml with the mobile phase.

Reference solution. Add 4 ml of solution A to 4 ml of a 0.5 per cent w/v solution of *cyclopentolate hydrochloride IPRS* in *water* and dilute to 10.0 ml with the mobile phase.

Chromatographic system

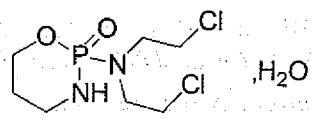
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 45 volumes of *0.2 M sodium dihydrogen orthophosphate* and 55 volumes of *methanol*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to cyclopentolate hydrochloride and the internal standard is not less than 4.0.

Inject the reference solution, test solution (a) and (b).

Calculate the content of $C_{17}H_{25}NO_3$, HCl in the eye drops.

Cyclophosphamide



$,H_2O$

$C_7H_{15}Cl_2N_2O_2P,H_2O$ Mol. Wt. 279.1

Cyclophosphamide is (*RS*)-2-bis(2-chloro-ethyl) amino-perhydro-1,3,2-oxazaphosphorinane 2-oxide monohydrate.

Cyclophosphamide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_{15}Cl_2N_2O_2P$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclophosphamide IPRS* or with the reference spectrum of cyclophosphamide.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve 0.1 g in 10 ml of *water* and add 5 ml of *silver nitrate solution*; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in *dilute nitric acid* but is soluble in *dilute ammonia solution* from which it can be reprecipitated by the addition of *dilute nitric acid*.

D. Dissolve 0.1 g in 3 ml of *nitric acid* and 1 ml of *sulphuric acid*, heat till brown fumes are evolved and the solution becomes colourless. Cool, add 10 ml of *water*, heat again up to 60° and add 10 ml of *ammonium molybdate solution*; a bright yellow precipitate is slowly formed.

Tests

Appearance of solution. A 2.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *2-butanone*, 12 volumes of *water*, 4 volumes of *acetone* and 2 volumes of *anhydrous formic acid*.

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent)*.

Test solution (b). Dilute 5 ml of test solution (a) to 50.0 ml with *ethanol (95 per cent)*.

Reference solution (a). Dilute 5 ml of test solution (b) to 50.0 ml with *ethanol (95 per cent)*.

Reference solution (b). A 0.2 per cent w/v solution of *cyclophosphamide IPRS* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. Place the plate while hot in a tank in which is placed a dish containing equal volumes of a 5 per cent w/v solution of *potassium permanganate* and *hydrochloric acid*, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a faint blue colour with *potassium iodide* and *starch solution*; do not expose long to cold air. Spray the plate with potassium iodide and starch solution and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot remaining on the line of application.

Heavy metals (2.3.13). 1.0 g dissolved in 2 ml of *dilute acetic acid* and diluted to 25 ml with *water* complies with the limit test for heavy metals, Method A (20 ppm).

Chlorides (2.3.12). A freshly prepared solution of 0.75 g in sufficient *water* to produce 25 ml complies with the limit test for chlorides (330 ppm).

Water (2.3.43). 5.8 to 7.0 per cent, determined on 0.3 g.

Assay. Weigh 0.1 g and dissolve in 50 ml of a 0.1 per cent w/v solution of *sodium hydroxide* in *ethylene glycol*; boil under a reflux condenser for 30 minutes and allow to cool. Rinse the condenser with 25 ml of *water*, add 75 ml of *2-propanol*, 15 ml of *2 M nitric acid*, 10.0 ml of *0.1 M silver nitrate* and 2 ml of *ferric ammonium sulphate solution* and titrate with *0.1 M ammonium thiocyanate*. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.01305 g of $C_7H_{15}Cl_2N_2O_2P$.

Cyclophosphamide Phosphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

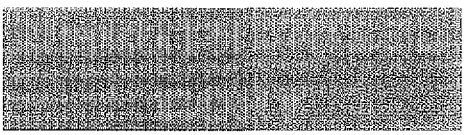
Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of cyclophosphamide.

Cyclophosphamide Phosphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.

Sterlity. Complies with the test for sterility (2.2.11).

Storage. Store at a temperature not exceeding 30°. Avoid long exposure to temperatures above 30°.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.



Cyclophosphamide Injection

Cyclophosphamide Injection is a sterile material consisting of Cyclophosphamide with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cyclophosphamide Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cyclophosphamide, $C_7H_{15}Cl_2N_2O_2P$.

Usual strengths. 100 mg; 200 mg; 500 mg; 1 g.

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Shake a quantity containing 0.2 g of anhydrous cyclophosphamide with 2 ml of *chloroform* and filter. The solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclophosphamide IPRS* or with the reference spectrum of cyclophosphamide.

B. Extract a quantity containing 0.2 g of anhydrous cyclophosphamide with *ether* and evaporate the extract to dryness. Reserve a portion of the residue for identification test C. Dissolve 0.1 g in 10 ml of *water* and add 5 ml of *silver nitrate solution*; no precipitate is produced. Boil; a white precipitate is produced which is insoluble in *dilute nitric acid* but is soluble in *dilute ammonia solution* from which it can be reprecipitated by the addition of *dilute nitric acid*.

C. Dissolve 0.1 g of the residue from test B in 3 ml of *nitric acid* and 1 ml of *sulphuric acid*, heat till brown fumes are evolved and the solution becomes colourless. Cool, add 10 ml of *water*, heat again up to 60° and add 10 ml of *ammonium molybdate solution*; a bright yellow precipitate is slowly formed.

Tests

pH (2.4.24). 3.0 to 6.0, determined in a 2.0 per cent w/v solution immediately after preparation.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *2-butanone*, 12 volumes of *water*, 4 volumes of *acetone* and 2 volumes of *anhydrous formic acid*.

Test solution. Dissolve a quantity of the contents of the sealed container containing 0.2 g of anhydrous cyclophosphamide in sufficient *ethanol (95 per cent)* to produce 10 ml and filter.

Reference solution. Dilute 1 volume of solution (1) to 100 volumes with *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. Place the plate while hot in a tank in which is placed a dish containing equal volumes of a 5 per cent w/v solution of *potassium permanganate* and *hydrochloric acid*, close the tank and allow to stand for 2 minutes. Remove the plate and place it in a current of cold air until excess chlorine is removed and an area of coating below the line of application gives not more than a faint blue colour with *potassium iodide* and *starch solution*; do not expose long to cold air. Spray the plate with *potassium iodide* and *starch solution* and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of cyclophosphamide.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Determine the weight of the content of 10 containers. Weigh accurately a suitable quantity of the mixed contents of the 10 containers containing about 50 mg of Cyclophosphamide, dissolve in *water* and dilute to 100.0 ml with *water*.

Reference solution (a). A 0.05 per cent w/v solution of *cyclophosphamide IPRS* in *water*.

Reference solution (b). Dissolve 25 mg of *cyclophosphamide IPRS* in 25 ml of *water*, add 5.0 ml of ethylparaben solution prepared by dissolving 185 mg of ethylparaben in 250 ml of *ethanol (95 per cent)* and dilute to 1000 ml with *water*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 195 nm,
- injection volume: 25 µl.

The relative retention time with reference to ethylparaben for cyclophosphamide is about 0.7.

Inject reference solution (b). The test is not valid unless the resolution between cyclophosphamide and ethylparaben is not less than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in the injection.

Storage. Store at a temperature not exceeding 30°. Avoid long exposure to temperatures above 30°. The solution should be used immediately after preparation as it deteriorates on storage.

Labelling. The label states (1) the quantity of Cyclophosphamide in terms of the equivalent amount of anhydrous cyclophosphamide; (2) the volume of Water for Injections to be added; (3) that the solution should be used immediately after preparation.

Cyclophosphamide Tablets

Cyclophosphamide Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous cyclophosphamide, $C_7H_{15}Cl_2N_2O_2P$. The tablets are coated.

Usual strengths. 10 mg; 50 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.2 g of anhydrous cyclophosphamide with 2 ml of *chloroform* and filter. The resulting solution complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyclophosphamide IPRS* or with the reference spectrum of cyclophosphamide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.001 per cent w/v solution of *cyclophosphamide IPRS* in the dissolution medium.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 195 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_7H_{15}Cl_2N_2O_2P$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse one intact tablet in 30 ml of *water* with the aid of ultrasound for 15 minutes with intermittent shaking, dilute to 50.0 ml with *water* and filter. Further, dilute quantitatively with *water* to obtain a solution of about the same concentration as the reference solution.

Reference solution (a). A 0.02 per cent w/v solution of *cyclophosphamide IPRS* in *water*.

Inject reference solution (a) and the test solution.

Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 50 mg of Cyclophosphamide in *water*, with the aid of ultrasound for 30 minutes with intermittent shaking and dilute to 100.0 ml with *water* and filter.

Reference solution (a). A 0.05 per cent w/v solution of *cyclophosphamide IPRS* in *water*.

Reference solution (b). Dissolve 18.5 mg of *ethylparaben* in 25 ml of *ethanol* and dilute to 100.0 ml with *water*.

Reference solution (c). Dissolve 25 mg of *cyclophosphamide IPRS* in 25 ml of *water*, add 5.0 ml of reference solution (b) and dilute to 50.0 with *water*.

Use the chromatographic system as described under Dissolution with the following modification
- injection volume: 25 µl.

Inject reference solution (a) and (c). The test is not valid unless resolution between the peaks due to cyclophosphamide and ethylparaben is not less than 2.0 in the chromatogram obtain with reference solution (c) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtain with reference solution (a).

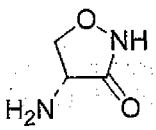
Inject reference solution (a) and the test solution.

Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous cyclophosphamide.

Cycloserine



$C_3H_6N_2O_2$ Mol. Wt. 102.1

Cycloserine is (*R*)-4-aminoisoxazolidin-3-one, an antimicrobial substance produced by the growth of certain strains of *Streptomyces orchidaceous* or *S. garyphalus* or obtained by synthesis.

Cycloserine contains not less than 98.0 per cent and not more than 100.5 per cent of $C_3H_6N_2O_2$, calculated on the dried basis.

Category. Antibacterial.

Description. A white or pale yellow, crystalline powder; hygroscopic.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. To 1 ml of a 0.01 per cent w/v solution in *0.1 M sodium hydroxide* add 3 ml of *1 M acetic acid* and 1 ml of a freshly prepared mixture of equal volumes of a 4 per cent w/v solution of *sodium nitroprusside* and *5 M sodium hydroxide*; a blue colour is produced slowly.

Tests

pH (2.4.24). 5.5 to 6.5, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +108° to +114°, determined in a 5.0 per cent w/v solution in *2 M sodium hydroxide*.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Condensation products. Absorbance of a 0.04 per cent w/v solution in *0.1 M sodium hydroxide* at about 285 nm, not more than 0.32 (2.4.7).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Dissolve about 10 mg of the substance under examination in 20.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve 10 mg of the *cycloserine IPRS* in 20.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Wakosil C8 RS),
- mobile phase: 0.1 per cent w/v of *methane sulphonic acid* and 0.78 per cent w/v of *sodium dihydrogen orthophosphate* in *water*, adjusted to pH 6.0 with *dilute sodium hydroxide* and filtered,
- flow rate: 1 ml per minute,
- spectrophotometer set at 227 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_3H_6N_2O_2$.

Storage. Store protected from moisture

Cycloserine Capsules

Cycloserine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cycloserine, $C_3H_6N_2O_2$.

Usual strength. 250 mg.

Identification

A. Shake a quantity of the contents of the capsules containing 10 mg of Cycloserine with 100 ml of *0.1 M sodium hydroxide* and filter. To 1 ml of the filtrate add 3 ml of *1 M acetic acid* and 1 ml of a freshly prepared mixture of equal volumes of a 4 per cent w/v solution of *sodium nitroprusside* and *5 M sodium hydroxide*; a blue colour is produced slowly.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate diluted to produce a 0.028 per cent w/v solution.

Reference solution. A 0.028 per cent w/v solution of *cycloserine IPRS* in the dissolution medium.

Use the chromatographic system described under Assay.

Calculate the content of $C_3H_6N_2O_2$.

Q. Not less than 80 per cent of the stated amount of $C_3H_6N_2O_2$.

Condensation products. Weigh the contents of the capsules containing about 0.5 g of Cycloserine, dissolve in 250 ml of *0.1 M sodium hydroxide* solution, disperse with the aid of ultrasound for 5 minutes. Dilute 5 ml of the solution to 25 ml with *0.1 M sodium hydroxide*. Absorbance of the resulting solution at about 285 nm, not more than 0.32 (2.4.7).

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g of the contents of the capsules, by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 20 capsules. Weigh accurately a quantity of the mixed contents of the capsules containing about 250.0 mg of Cycloserine dissolve in *phosphate buffer pH 6.8,* dilute to 250.0 ml with the same solvent and filter. Dilute 5.0 ml of the filtrate to 25.0 ml with *phosphate buffer pH 6.8.*

Reference solution. A 0.02 per cent w/v solution of *cycloserine IPRS* in *phosphate buffer pH 6.8.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Warkosil C8 RS),
- mobile phase: dissolve 1.0 g of *methane sulphonic acid* and 7.8 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 ml of *water* and adjusted to pH 6.0 with *dilute sodium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 227 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_3H_6N_2O_2$ in the capsules.

Storage. Store protected from moisture.

Cycloserine Tablets

Cycloserine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of cycloserine, $C_3H_6N_2O_2$.

Usual strengths. 125 mg; 250 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.5 g of Cycloserine with 25 ml of *0.1 M sodium hydroxide* for 5 minutes and filter. The optical rotation of the filtrate is about +2.2°(2.4.22).

B. To 0.2 ml of the filtrate obtained in test A add 3 ml of *1 M acetic acid* and 1 ml of a freshly prepared mixture of equal volumes of a 4 per cent w/v solution of *sodium nitroprusside* and *5 M sodium hydroxide*; a blue colour is produced slowly.

Tests

Light absorption. Shake a quantity of the powdered tablets containing 0.25 g of Cycloserine with 80 ml of *0.1 M sodium hydroxide* for 10 minutes, add sufficient *0.1 M sodium hydroxide* to produce 100.0 ml, mix and filter. Dilute a suitable volume of the filtrate with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.0025 per cent w/v of Cycloserine. Absorbance of the resulting solution, measured within 15 minutes of preparing the final solution, at the maximum at about 219 nm, 0.78 to 0.96 (2.4.7).

Disintegration (2.5.1). Not more than 30 minutes.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g of the powdered tablets, by drying in an oven at about 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Other tests. Comply with the tests stated under Tablets.

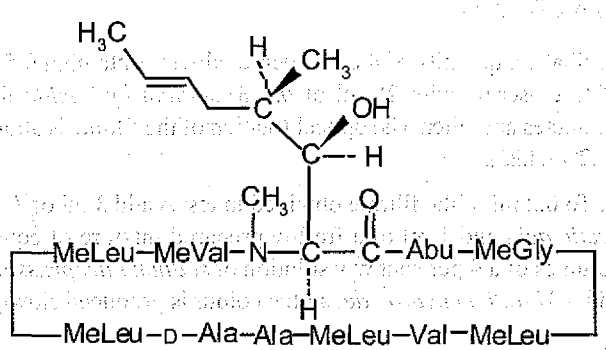
Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.1 g of Cycloserine, shake with 150 ml of *water* for 30 minutes, add sufficient *water* to produce 200.0 ml and filter. To 10.0 ml of the filtrate add

10 ml of *water* and 25 ml of *0.2 M sodium hydroxide*, dilute to 50.0 ml with *water* and mix. To 4.0 ml of the mixture add 10 ml of *1 M acetic acid* and 4 ml of *sodium nitroprusside solution*, dilute to 20 ml with *1 M acetic acid*, mix and allow to stand for 15 minutes. Measure the absorbance of the resulting solution at the maximum at about 625 nm (2.4.7), using as the blank a solution prepared by treating 4.0 ml of *0.1 M sodium hydroxide* in the same manner beginning at the words "add 10 ml of *1 M acetic acid*..." Calculate the content of $C_3H_6N_2O_2$ from the absorbance obtained by repeating the operation using *cycloserine IPRS* in place of the powdered tablets.

Storage. Store at a temperature not exceeding 30°.

Cyclosporine

Cyclosporin A



$C_{62}H_{111}N_{11}O_{12}$ Mol. Wt. 1202.6

Cyclosporine is cyclo[[(*E*)-(2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl].

Cyclosporine contains not less than 97.0 per cent and not more than 101.5 per cent of cyclosporine A, $C_{62}H_{111}N_{11}O_{12}$, calculated on the dried basis.

Category. Immunosuppresant.

Description. A white to off-white powder.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 10.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent) and sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 0.1 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *cyclosporine IPRS* in the solvent mixture.

Reference solution (b). A 0.001 per cent w/v solution of *cyclosporine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 80°,
- mobile phase: a mixture of 52 volumes of *water*, 43 volumes of *acetonitrile*, 5 volumes of *tert-butyl methyl ether* and 0.1 volume of *orthophosphoric acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$.

Storage. Store protected from light and moisture.

Cyclosporine Capsules

Cyclosporine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyclosporine, $C_{62}H_{111}N_{11}O_{12}$.

Usual strengths. 25 mg; 50 mg; 100 mg; 250 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

For capsules containing liquid —

Apparatus No. 2 (Paddle),
Medium. 500 ml of *water*,
Speed and time. 50 rpm and 15 minutes.

Place 1 capsule in each vessel, and allow the capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

Tolerances — The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 minutes but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 minutes but not more than 30 minutes.

For capsules containing powder —

Apparatus No. 1 (Basket),
Medium. 1000 ml of *0.1 M hydrochloric acid* containing 0.5 per cent of *sodium lauryl sulphate*,
Speed and time. 150 rpm and 90 minutes.

Determine by liquid chromatography (2.4.14).

Test solution. Withdraw a suitable volume of the medium and filter. Dilute 5.0 ml of the solution to 10.0 ml with *acetonitrile*.

Reference solution. A solution of *cyclosporine IPRS* in the dissolution medium suitably diluted with the *acetonitrile* to obtain a solution having the same concentration as that of the test solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 80°,
- mobile phase: a mixture of 900 volumes of *acetonitrile*, 450 volumes of *water*, 50 volumes of *methanol* and 0.5 volume of *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 700 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$.

Q. Not less than 80 per cent of the stated amount of $C_{62}H_{111}N_{11}O_{12}$.

Water (2.3.43). Not more than 3.5 per cent w/w, for Capsules that contain powder, using finely ground Capsule contents.

Other tests. Comply with the tests stated under Capsules.

Assay

Where capsules contain liquid—

Test solution. Using a sharp blade, carefully cut open not fewer than 20 capsules, and with the aid of *ethanol* transfer the contents of the capsules to a suitable volumetric flask. Wash the blade with *ethanol*, and transfer the washings to the volumetric flask. Dilute the contents of the volumetric flask with *ethanol* to volume and mix. Quantitatively dilute an accurately measured volume of the solution with *ethanol* to obtain a solution having a concentration of 1 mg of cyclosporine per ml.

Reference solution. A 0.1 per cent w/v solution of *cyclosporine IPRS* in *ethanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with dimethylsilane bonded to porous silica (5 to 10 µm),
- column temperature: 70°,
- mobile phase: a mixture of 550 volumes of *acetonitrile*, 400 volumes of *water*, 50 volumes of *methanol* and 0.5 volume of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 700 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$.

Where capsules contain powder—

Solvent mixture. 9 volumes of *acetonitrile*, 5 volumes of *tetrahydrofuran* and 4 volumes of *ethanol*.

Test solution. Disperse a quantity of the mixed contents of 20 capsules containing 0.1 g of cyclosporine with 10 ml of *water* and 40 ml of the solvent mixture, dissolve with the aid of ultrasound, dilute to 100.0 ml with the solvent mixture and filter.

Reference solution. Disperse 25 mg of *cyclosporine IPRS* with 2.5 ml of *water* with the aid of ultrasound for 10 minutes. Add about 10 ml of the solvent mixture, sonicate for 5 minutes and dilute to 25.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with trimethylsilane bonded to porous silica (3 to 10 µm),
- column temperature: 70°,
- mobile phase: a mixture of 605 volumes of *acetonitrile*, 400 volumes of *water*, 50 volumes of *methanol* and 0.5 volume of *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 700 theoretical plates, tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$ in the cpasules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Cyclosporine Eye Drops

Cyclosporine Eye Drops is a sterile solution of Cyclosporine in a suitable vehicle.

Cyclosporine Eye Drops contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cyclosporine, $C_{62}H_{111}N_{11}O_{12}$.

Usual strengths. 0.05 per cent w/v; 0.1 per cent w/v.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the eye drops containing 20 mg of Cyclosporine to 100.0 ml with *methanol.*

Reference solution. A 0.02 per cent w/v solution of *cyclosporine IPRS* in *methanol.*

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with dimethylsilane bonded to porous silica (5 µm),
- column temperature: 70°,
- mobile phase: a mixture of 55 volumes of *acetonitrile*, 40 volumes of *water*, 5 volumes of *methanol* and 0.05 volume of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 700 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$ in the eye drops.

Storage. Store protected from light, at a temperature not exceeding 30°.

Cyclosporine Injection

Cyclosporine Injection is a sterile solution of Cyclosporine in a suitable vehicle.

Cyclosporine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyclosporine, $C_{62}H_{111}N_{11}O_{12}$.

Usual strengths. 50 mg per ml; 100 mg per ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase A. Ethyl ether.

Mobile phase B. A mixture of 60 volumes of *ethyl acetate*, 40 volumes of *methyl ethyl ketone*, 2 volumes of *water* and 1 volume of *formic acid.*

Spray reagent A. Mix 5 ml of 1.7 per cent w/v solution of *bismuth subnitrate* in 20 per cent v/v solution of *acetic acid*, 5 ml of 40 per cent w/v solution of *potassium iodide* and 20 ml of *glacial acetic acid*, dilute to 100 ml with *water.*

Spray reagent B. Hydrogen peroxide solution (10 vol).

Test solution. Dilute a volume of the injection containing 50 mg of Cyclosporine to 100.0 ml with *methanol.*

Reference solution. A 0.05 per cent w/v solution of *cyclosporine IPRS* in *methanol.*

Apply to the plate 10 µl of each solution. Allow the spots to dry in a current of air. Develop the chromatogram using mobile phase A to three fourth length of the plate. Dry the plate in air until the odour of the solvent is no longer detectable. Place the plate in second chromatographic chamber and develop the chromatogram using mobile phase B until the solvent front has moved about three fourth length of the plate. Again dry the plate in hot air and spray the plate with spray reagent A, immediately. Again spray plate with spray reagent B. The principal spot at Rf value of about 0.45 in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Ethanol *(if present).* 80.0 to 120.0 per cent of the labelled amount.

Determine by gas chromatography (2.4.13).

Internal standard solution. A 6 per cent v/v solution of *n-propyl alcohol* in *butyl alcohol.*

Test solution. Transfer a volume of the injection containing 0.256 g of *ethanol* to 20-ml volumetric flask, add 5.0 ml of the internal standard solution and dilute to volume with *butyl alcohol.*

Reference solution. Transfer 0.256 g of *ethanol* to 20-ml volumetric flask, add 5.0 ml of the internal standard solution and dilute to volume with *butyl alcohol.*

Chromatographic system

- a glass column 2 m x 2 mm, packed with ethylvinylbenzene-divinylbenzene copolymer, having a nominal surface area 500 to 600 m^2 per g and an average pore diameter of 0.0075 µm,
- temperature:
  column 145° for 8 minutes, 145° to 270° @ 32° per minute, inlet port. 280° and detector. 290°,
- a flame ionisation detector,
- flow rate: 35 ml per minute, using nitrogen as the carrier gas.
- injection volume: 1 µl.

The elution order is ethanol, n-propyl alcohol and butyl alcohol.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections from the peak area ratio of the ethanol to n-propyl alcohol is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of ethanol from the peak area ratio of the ethanol to n-propyl alcohol in the chromatograms obtained with the reference solution and the test solution.

Sterility (2.2.11). Complies with the test for sterility.

Bacterial endotoxins (2.2.3). Not more than 0.84 Endotoxin Unit per mg of cyclosporine.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volumes of the injection in *methanol* to obtain a solution containing 0.05 per cent w/v of Cyclosporine.

Reference solution. A 0.05 per cent w/v solution of *cyclosporine IPRS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with dimethylsilane bonded to porous silica (5 µm),
- column temperature: 70°,
- mobile phase: a mixture of 55 volumes of *acetonitrile,* 40 volumes of *water*, 5 volumes of *methanol* and 0.05 volume of *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless, the column efficiency is not less than 700 theoretical plates, the tailing factoris not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$ in the injection.

Storage. Store at a temperature not exceeding 30°, in single dose or multiple-dose container.

Labelling. The label states that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.

Cyclosporine Oral Solution

Cyclosporine Oral Solution is a solution of Cyclosporine in a suitable vehicle.

Cyclosporine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyclosporine, $C_{62}H_{111}N_{11}O_{12}$.

Usual strength. 100 mg per ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 80 volumes of *methanol* and 20 volumes of *chloroform*.

Test solution. Dilute a volume of the oral solution with the solvent mixture to obtain a 0.1 per cent w/v solution of Cyclosporine.

Reference solution. A 0.1 per cent w/v solution of *cyclosporine IPRS* in the solvent mixture.

Mobile phase A. *Ethyl ether,*

Mobile phase B. A mixture of 60 volumes of *ethyl acetate,* 40 volumes of *methyl ethyl ketone,* 2 volumes of *water* and 1 volume of *formic acid.*

Spray reagent A. Mix 5 ml each of 1.7 per cent w/v solution of *bismuth subnitrate* in 20 per cent w/v solution of *acetic acid* and 40.0 per cent w/v solution of *potassium iodide* and 20 ml of *glacial acetic acid,* and dilute with *water* to 100.0 ml. Prepare freshly.

Spray reagent B. Hydrogen peroxide solution (10 volume).

Apply to the plate 10 µl of the reference solution and the test solution. After development, dry the plate in a current of air, place the plate in a suitable chromatographic chamber, and develop the chromatogram, using mobile phase A, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow it to dry. Place the plate in a second chromatographic chamber, and develop the chromatogram in mobile phase B until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to dry. Spray the plate with spray reagent A. Immediately again spray the plate with spray reagent B. Cyclosporine appears as a brown spot having an Rf value of about 0.45.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Alcohol *(if present).* 80.0 per cent w/w to 120.0 per cent w/w, determined by gas chromatography (2.4.13).

Internal standard. A mixture of 3 volumes of *n-propyl alcohol* and 50 volumes of *butyl alcohol.*

Test solution. Transfer a suitable quantity of oral solution to a suitable volumetric flask. Add 24 per cent *internal standard solution* of the final volume, and dilute with *butyl alcohol* to obtain a 1.0 per cent w/v solution of alcohol.

Reference solution (a). A 5.0 per cent w/v solution of *ethanol* in *butyl alcohol.*

Reference solution (b). Transfer a suitable quantity of reference solution (a) to a suitable volumetric flask. Add 24 per cent *internal standard solution* of the final volume, and dilute with *butyl alcohol* to obtain a 1.0 per cent w/v solution of alcohol.

Chromatographic system

- a glass column 2.0 m × 2 mm, packed with support (copolymer of ethylvinylbenzene and divinylbenzene, nominal surface area 500 to 600 m^2 per g, average pore diameter 0.0076 µm),
- temperature:

column	time (min.)	temperature (°)
	0-8	145
	8-12	145→270

- inlet port: 280°,
- flame ionisation detector at 290°,
- flow rate: 35 ml per minute, using nitrogen as the carrier gas.

The elution order of the peaks is alcohol, *n*-propyl alcohol, and butyl alcohol.

Inject 1 µl of reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject 1 µl each of reference solution (b) and the test solution.

Calculate the content of C_2H_5O H in the oral solution.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *methanol* and 20 volumes of *chloroform.*

Test solution. Dilute a volume of the oral solution with the solvent mixture to obtain 0.1 per cent w/v solution of Cyclosporine. Use the solution immediately after preparation.

Reference solution. A 0.1 per cent w/v solution of *cyclosporine IPRS* in the solvent mixture. Use the solution immediately after preparation.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with dimethylsilane bonded to porous silica (5 to 10 µm),
- column temperature: 50°,
- mobile phase : a mixture of 55 volumes of *acetonitrile,* 5 volumes of *methanol,* 40 volumes of *water* and 0.05 volume of *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

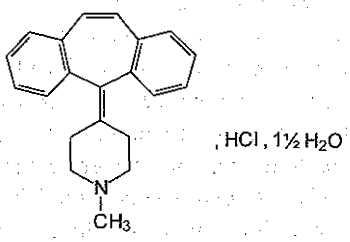
Inject the reference solution. The test is not valid unless the column efficiency is not less than 700 theoretical plates, tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{62}H_{111}N_{11}O_{12}$ in the oral solution.

Storage. Store protected from moisture.

Cyproheptadine Hydrochloride



$C_{21}H_{21}N, HCl, 1½H_2O$ Mol. Wt. 350.9

Cyproheptadine Hydrochloride is 4-(5*H*-dibenzo[*a,d*]-cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride sesquihydrate.

Cyproheptadine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{21}H_{21}N,HCl$, calculated on anhydrous basis.

Category. Histamine H_1-receptor antagonist.

Description. A white or slightly yellow, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Dissolve 0.1 g in 10 ml of water, make alkaline with *1 M sodium hydroxide*, extract with 5 ml of *dichloromethane*, dry over *anhydrous sodium sulphate* and remove the solvent with the aid of a current of nitrogen. The oily residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyproheptadine hydrochloride IPRS* treated in the same manner or with the reference spectrum of cyproheptadine.

B. When examined in the range 230 nm to 360 nm, a 0.002 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum only at about 286 nm, about 0.67 (2.4.7).

C. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *cyclohexane*, 20 volumes of *ether* and 5 volumes of *diethylamine*.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.1 per cent w/v solution of *cyproheptadine hydrochloride IPRS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v, each of, *imipramine hydrochloride IPRS* and *cyproheptadine hydrochloride IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

D. A saturated solution gives reaction A of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in mobile phase A and dilute to 20.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution (b). Dissolve 2 mg each of *cyproheptadine impurity A IPRS, cyproheptadine impurity B IPRS* and *cyproheptadine impurity C IPRS* in mobile phase A, add 1.0 ml of the test solution and dilute to 100.0 ml with mobile phase A.

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 10.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 60 volumes of a solution prepared by dissolving 6.12 g of *potassium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH 4.5 with *orthophosphoric acid* and dilute to 1000 ml with *water* and 40 volumes of *acetonitrile*,

  B. a mixture of 40 volumes of a solution prepared by dissolving 6.12 g of *potassium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH 4.5 with *orthophosphoric acid* and dilute to 1000 ml with *water* and 60 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,

– flow rate: 1 ml per minute,
– spectrophotometer set at 230 nm,
– injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
10	100	0
10.1	0	100
35	0	100
36	100	0
45	100	0

Name	Relative retention time
Cyproheptadine impurity C[1]	0.7
Cyproheptadine (Retention time: about 8 minutes)	1.0
Cyproheptadine impurity B[2]	2.6
Cyproheptadine impurity A[3]	3.9

[1]5-(1-methylpiperidin-4-yl)-5*H*-dibenzo[*a*,*d*][7]annulen-5-ol,

[2]dibenzosuberone,

[3]dibenzocycloheptene.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cyproheptadine impurity C and cyproheptadine is not less than 7.0.

Inject reference solution (a), (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to each cyproheptadine impurities A, B, C is not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 7.0 to 9.0 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.5 g, dissolve in 0.5 ml of *acetic anhydride* and 20 ml of *anhydrous glacial acetic acid* and add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03239 g of $C_{21}H_{21}N,HCl$.

Storage. Store protected from light.

Cyproheptadine Syrup

Cyproheptadine Hydrochloride Syrup

Cyproheptadine Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyproheptadine hydrochloride, $C_{21}H_{21}N,HCl$.

Usual strength. 2 mg in 5 ml.

Identification

To 5 ml add 5 ml of a 1 per cent w/v solution of *sodium bicarbonate* and extract with three quantities, each of 15 ml, of *2,2,4-trimethylpentane*. Wash the combined 2,2,4-trimethylpentane extracts with 5 ml of the *sodium bicarbonate solution* and discard the washings. Evaporate the *2,2,4-trimethylpentane solution* to dryness on a water-bath and dissolve the residue in 100 ml of *ethanol (95 per cent)*. When examined in the range 230 nm to 360 nm, the resulting solution shows an absorption maximum only at about 286 nm (2.4.7).

Tests

pH (2.4.24). 3.5 to 4.5.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the syrup containing 2 mg of Cyproheptadine Hydrochloride in the mobile phase and dilute to 100.0 ml with the mobile phase and filter.

Reference solution. A 0.002 per cent w/v solution of *cyproheptadine hydrochloride IPRS* in the mobile phase.

Chromatographic system
– a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
– mobile phase: a mixture of 65 volumes of 0.3 per cent v/v *methanesulphonic acid*, 20 volumes of *acetonitrile*, and 15 volumes of *isopropyl alcohol* adjusted to pH 4.0 with *triethylamine*,
– flow rate: 1 ml per minute,
– spectrophotometer set at 285 nm,
– injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml (2.4.29) of the syrup and calculate the content of $C_{21}H_{21}N,HCl$, weight in volume.

Storage. Store protected from light and moisture.

Cyproheptadine Tablets

Cyproheptadine Hydrochloride Tablets

Cyproheptadine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cyproheptadine hydrochloride, $C_{21}H_{21}N,HCl$.

Usual strength. 4 mg.

Identification

A. To a quantity of the powdered tablets containing 20 mg of Cyproheptadine Hydrochloride add 10 ml of *water* and 2.5 ml of *0.1 M sodium hydroxide*, extract with 10 ml of *dichloromethane*, filter through *anhydrous sodium sulphate* placed over absorbent cotton moistened with *dichloromethane* and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyproheptadine hydrochloride IPRS* treated in the same manner or with the reference spectrum of cyproheptadine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Extract a quantity of the powdered tablets containing 20 mg of Cyproheptadine Hydrochloride with 7 ml of *water*, filter, add 0.3 ml of *5 M ammonia* to the filtrate and filter again. The filtrate gives reaction A of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate suitably diluted with the medium, if necessary, at the maximum at about 285 nm (2.4.7). Calculate the content of $C_{21}H_{21}N,HCl$ in the medium from the absorbance obtained from a solution of known concentration of *cyproheptadine hydrochloride IPRS* in the dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{21}H_{21}N,HCl$.

Related substances. Determine by thin-layer chromatography (2.4.17), using a precoated silica gel plate.

Mobile phase. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

Test solution (a). Shake mechanically for 10 minutes a quantity of the powdered tablets containing 50 mg of Cyproheptadine Hydrochloride with 5 ml of the mobile phase and filter (such as Whatman GF/C filter paper).

Test solution (b). Dilute 1 volume of test solution (a) to 10 volumes with the mobile phase.

Reference solution (a). Dilute 1 volume of test solution (a) serially in two steps to 1000 volumes with the mobile phase.

Reference solution (b). A solution containing 0.1 per cent w/v of *cyproheptadine hydrochloride IPRS* in the mobile phase.

Reference solution (c). A solution containing 0.002 per cent w/v of *dibenzocycloheptatriene IPRS* in the mobile phase.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *ethanolic sulphuric acid (20 per cent)*. Heat at 110° for 30 minutes and examine under ultraviolet light at 365 nm. In the chromatogram obtained with test solution (a) any spot corresponding to dibenzocycloheptatriene is not more intense than the spot in the chromatogram obtained with reference solution (c) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following test solution.

Test solution. Disperse one tablet in the mobile phase and dilute to 50.0 ml with the mobile phase.

Calculate the content of $C_{21}H_{21}N,HCl$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 80 mg of Cyproheptadine Hydrochloride in 500 ml of the mobile phase, with the aid of ultrasound for 15 minutes and agitate for 30 minutes and dilute to 1000.0 ml with the mobile phase, filter.

Reference solution. A 0.008 per cent w/v solution of *cyproheptadine hydrochloride IPRS* in the mobile phase.

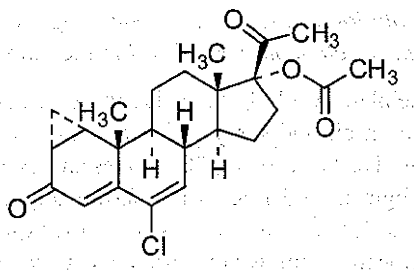
Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a 0.3 per cent v/v *methanesulphonic acid*, 20 volumes of *acetonitrile*, and 15 volumes of *isopropyl alcohol* adjusted to pH 4.0 with *triethylamine*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{21}N,HCl$ in the tablets.

Cyproterone Acetate



$C_{24}H_{29}ClO_4$ Mol. Wt. 416.9

Cyproterone Acetate is (1*β*, 2*β*)-6-chloro-1,2-dihydro-17-acetyloxy-3'*H*-cyclopropa[1,2]pregna-1,4,6-triene-3,20-dione.

Cyproterone Acetate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{24}H_{29}ClO_4$, calculated on the dried basis.

Category. Anticancer.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyproterone acetate IPRS* or with the reference spectrum of cyproterone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. a mixture of equal volumes of *cyclohexane* and *ethyl acetate*.

Test solution. Dissolve 0.02 g of the substance under examination in *dichloromethane* and dilute to 10 ml with the same solvent.

Reference solution. A 0.2 per cent w/v solution of *cyproterone acetate IPRS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve about 1.0 mg with 2 ml of *sulphuric acid* and heat on a water-bath for 2 minutes. A red colour develops. Cool and add the solution cautiously to 4 ml of *water* and shake. The solution becomes violet.

D. Incinerate about 30 mg with 0.3 g of *anhydrous sodium carbonate* over a naked flame for about 10 minutes. Cool, dissolve the residue in 5 ml of *dilute nitric acid* and filter. To 1 ml of the filtrate, add 1 ml of *water*. The solution gives reaction A of chlorides (2.3.1).

E. It gives the reactions of acetyl groups (2.3.1).

Tests

Specific optical rotation (2.4.22). +152° to +157°, determined in a 1.0 per cent w/v solution in *acetone*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in *acetonitrile* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

Reference solution (b). Dissolve 5 mg of *medroxy-progesterone acetate IPRS* in *acetonitrile* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with reference solution (a).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cyproterone acetate and medroxyprogesterone acetate is not less than 3.0.

Inject reference solution (a) and the test solution. Run the chromatogram at least twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 80° at a pressure not exceeding 0.7 kPa.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh accurately about 0.05 g, dissolve in *methanol* and dilute to 50.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 282 nm (2.4.7). Calculate the content of $C_{24}H_{29}ClO_4$ taking 414 as the specific absorbance at 282 nm.

Storage. Store protected from light.

Cyproterone Tablets

Cyproterone Acetate Tablets

Cyproterone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cyproterone acetate, $C_{24}H_{29}ClO_4$.

Usual strength. 50 mg.

Identification

A. Shake a quantity of powdered tablets containing about 100 mg of cyproterone acetate with 20 ml of *dichloromethane*, filter and evaporate the filtrate to dryness using a rotary evaporator and a water-bath at 40°. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cyproterone acetate IPRS* or with the reference spectrum of cyproterone acetate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 0.07 per cent w/v solution of *sodium dodecyl sulphate* in *0.1M hydrochloric acid* (For tablets containing less than 100 mg of Cyproterone Acetate); 900 ml of 0.1 per cent w/v solution of *sodium dodecyl sulphate* in *0.1M hydrochloric acid* (For tablets containing more than 100 mg of Cyproterone Acetate),
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary with dissolution medium at 285 nm (2.4.7). Calculate the content of cyproterone acetate, $C_{24}H_{29}ClO_4$ in the medium from the absorbances obtained from a solution of known concentration of *cyproterone acetate IPRS*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *acetonitrile* and 20 volumes of *water*.

Test solution. Shake a quantity of powdered tablets containing about 0.1 g of Cyproterone Acetate in 50 ml of the solvent mixture for 5 minutes, dilute to 100 ml with *water* and filter.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v, each of, *cyproterone acetate IPRS* and *medroxyprogesterone acetate IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (Such as Spherisorb ODS 2),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cyproterone acetate and medroxyprogesterone acetate is not less than 3.0.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of powdered tablets containing about 50 mg of Cyproterone Acetate in 100 ml of the solvent mixture and filter. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *cyproterone acetate IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v, each of, *cyproterone acetate IPRS* and *medroxyprogesterone*

acetate IPRS in the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Use the chromatographic system as described under Related substances.

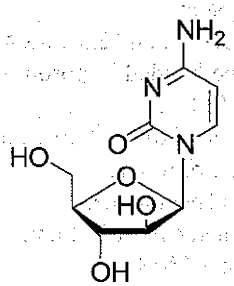
Inject reference solution (b). The test is not valid unless the resolution between the peak due to cyproterone and medroxyprogesterone is not less than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{24}H_{29}ClO_4$ in the tablets.

Cytarabine

β-Cytosine Arabinoside



$C_9H_{13}N_3O_5$ Mol. Wt. 243.2

Cytarabine is 1-β-D-arabinofuranosylcytosine.

Cytarabine contains not less than 99.0 per cent and not more than 100.5 per cent of $C_9H_{13}N_3O_5$, calculated on the dried basis.

Category. Anticancer.

Description. A white or almost white, crystalline powder.

CAUTION — Cytarabine is very poisonous. Great care should be taken to avoid inhaling the particles of cytarabine and exposing the skin to the dried substance.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cytarabine IPRS* or with the reference spectrum of cytarabine.

B. When examined in the range 230 nm to 360 nm, a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 280 nm, about 0.55 (2.4.7).

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Specific optical rotation (2.4.22). +154° to +160°, determined in a 1.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 65 volumes of *2-butanone*, 20 volumes of *acetone* and 15 volumes of *water*.

Test solution (a). A 5 per cent w/v solution of the substance under examination in *water*.

Test solution (b). A 0.2 per cent w/v solution of the substance under examination in *water*.

Reference solution (a). A 0.025 per cent w/v solution of the substance under examination in *water*.

Reference solution (b). A 0.2 per cent w/v solution of *cytarabine IPRS* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 60° for 3 hours at a pressure of 0.2 kPa to 0.7 kPa.

Assay. Weigh accurately about 0.5 g and dissolve in 40 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02432 g of $C_9H_{13}N_3O_5$.

Cytarabine intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.07 Endotoxin Unit per mg.

Cytarabine intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store protected from light. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labeling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Cytarabine Injection

β-Cytosine Arabinoside Injection

Cytarabine Injection is a sterile material consisting of Cytarabine with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Cytarabine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of cytarabine, $C_9H_{13}N_3O_5$.

Usual strengths. 100 mg per vial; 500 mg per vial; 1 g per vial.

Description. A white or almost white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

Mix a qunatity of injection containing 0.1 g of cytarabine with 10 ml of hot *ethanol (95 per cent)*, filter, allow the filtrate to cool and induce crystallisation if necessary. Filter, wash the crystals with 2 ml of *ethanol (95 per cent)* and dry at 60° at a pressure of 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cytarabine IPRS* or with the reference spectrum of cytarabine.

Tests

pH (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution in the solvent stated on the label.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 65 volumes of *2-butanone*, 20 volumes of *acetone* and 15 volumes of *water*.

Test solution. Dissolve a quantity of injection to obtain a solution containing 4.0 per cent w/v of cytarabine in *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with *water*.

Reference solution (b). A solution containing 0.04 per cent w/v solution of *uridine* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution with an R_f value of about 1.1 relative to the spot in the chromatogram obtained with reference solution (b) is not more intense than the spot in the chromatogram obtained with reference solution (b). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Water (2.3.43). Not more than 3.0 per cent, determined on 0.8 g.

Bacterial endotoxins (2.2.3). Not more than 0.07 Endotoxin unit per mg.

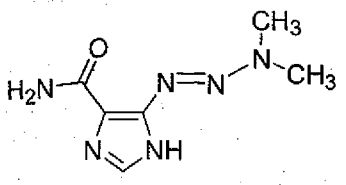
Assay. Determine the weight of the contents of 10 containers. Weigh accurately about 0.5 g of the mixed contents of the 10 containers and dissolve by heating, if necessary, in 80 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02432 g of $C_9H_{13}N_3O_5$.

Storage. Store protected from light.

D

Dacarbazine



$C_6H_{10}N_6O$ Mol Wt. 182.2

Dacarbazine is 5-[1E)-(3,3-dimethyltriaz-1-enyl)-1*H*-imidazole-4-carboxamide.

Dacarbazine contains not less than 98.5 per cent and not more than 101.0 per cent of $C_6H_{10}N_6O$, calculated on the anhydrous basis.

Category. Antineoplastic and immunomodulating alkylating agent.

Description. A white or slightly yellowish, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *dacarbazine IPRS* or with the reference spectrum of dacarbazine.

B. When examined in the range 200 nm to 400 nm, a 0.00075 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at 323 nm and a shoulder at 275 nm (2.4.7).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *glacial acetic acid*, 20 volumes of *water*, 50 volumes of *butanol*.

Test solution. A 0.04 per cent w/v solution of substances under examination in *methanol*.

Reference solution. A 0.04 per cent w/v solution of *dacarbazine IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. Dissolve 0.25 g in 21 per cent w/v solution of *citric acid* and dilute to 25.0 ml with the same solution. The solution is clear and not more intensely coloured than reference solution BYS6 (2.4.1).

Related substances. A. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use and protected from light.

Test solution. Dissolve 50 mg of the substance under examination and 75 mg of *citric acid* in *water* and dilute to 5.0 ml with the same solvent.

Reference solution(a). A 0.002 per cent w/v solution of *3,7-dihyro-4H-imidazol[4,5-d]-1,2,3-triazin-4-one IPRS* (dacarbazine impurity A) in *water*.

Reference solution(b). Dissolve 5.0 mg of *5-amino-1H-imidazole-4-carboxamide IPRS* (dacarbazine impurity B) in *water*, add 0.5 ml of the test solution and dilute to 10.0 ml with *water*. Dilute 1.0 ml of the solution to 50.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution of 1.563 per cent w/v of *glacial acetic acid* in *water* containing 0.233 per cent w/v of *sodium dioctyl sulphosuccinate*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 25 µl.

The retention time of dacarbazine impurity A is about 3 minutes.

Inject reference solution (a) and test solution. Run the chromatogram three times the retention time of dacarbazine impurity A. In the chromatogram obtained with the test solution, the area of any peak corresponding to dacarbazine impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

B. Determine by liquid chromatography (2.4.14).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm).
- mobile phase: A mixture of 45 volumes of 1.563 per cent w/v solution *of glacial acetic acid* containing 0.233 per cent w/v of *sodium dioctyl sulphosuccinate* and 55 volumes of *methanol*.
- flow rate: 1.2 ml per minute.
- spectrophotometer set at 254 nm.
- injection volume: 10 µl.

The relative retention time with reference to dacarbazine for *5-amino-1H-imidazole-4-carboxamide* (dacarbazine impurity B) is about 0.7.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to dacarbazine and dacarbazine impurity B is not less than 1.5.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to dacarbazine impurity B is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity D. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.2 g of the substance under examination in 5 µl of *water* and seal the vial.

Reference solution (a). Dilute 2.5 ml of *dimethylamine solution* (dacarbazine impurity D) to 100.0 ml with *water*. Transfer 10 µl of the solution in a vial and seal.

Reference solution (b). A mixture of 10 µl of reference solution (a) and 10 µl of 1 per cent w/v solution of *triethylamine* in sealed vial.

Chromatographic system

- a fused-silica capillary column 30 m x 0.53 mm, packed with base-deactivated polyethylene glycol (film thickness: 1.0 µm),
- temperature:

column	time (min.)	temperature (°)
	0-3	35
	3-11	35 → 165

- inlet port at 180° and detector at 220°,
- a flame ionisation detector,
- flow rate: 13 ml per minute, using helium or nitrogen as the carrier gas,
- split ratio of 1:1,
- injection volume 1ml.

Static head-space conditions which may be used:

- equilibration temperature 60°,
- equilibration time 10 minutes,
- transfer line temperature 90°,
- pressurisation time 30 seconds.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to triethylamine and dacarbazine impurity D is not less than 2.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to dacarbazine impurity D is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Dissolve 0.150 g in 30 ml of *anhydrous glacial acetic acid* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01822 g of $C_6H_{10}N_6O$.

Storage. Store protected from light, at a temperature between 2° to 8°.

Dacarbazine Injection

Dacarbazine Injection is a sterile material consisting of Dacarbazine with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of sealed container in the requisite amount of sterile water for Injections, immediately before use.

The constituted solution complies with the requirements for clarity of solution and particulate matter stated under parenteral preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Dacarbazine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dacarbazine, $C_6H_{10}N_6O$.

Usual strengths. 100 mg per vial; 200 mg per vial.

Description. A white or pale yellow powder.

The contents of the sealed container comply with the requirements stated under parenteral preparations (Powders for Injection) and with the following requirements.

Identification

A. When examined in the range of 210 nm to 360 nm, a 0.0006 per cent w/v solution of dacarbazine in *mixed phosphate buffer* pH 7.0, shows absorption maxima at 237 nm and 330 nm (2.4.7).

B. In the test for 5-aminoimidazole-4-carboxamide hydrochloride, the principal peak in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

Tests

5-Aminoimidazole-4-carboxamide hydrochloride. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve an accurately weighed quantity of powder containing 0.2 g of dacarbazine in 50.0 ml of *0.1 M acetic acid.*

Test solution (b). Dilute 1.0 ml of the test solution (a) to 100.0 ml with *0.1 M acetic acid.*

Reference solution (a). A 0.004 per cent w/v solution of *dacarbazine IPRS* in *0.1 M acetic acid.*

Reference solution (b). A 0.0024 per cent w/v solution of 5-aminoimidazole-4-carboxamide in *0.1 M acetic acid.*

Chromatographic system

- a stainless steel column 20 cm x 4 mm, packed with end-capped octadecylsilyl silica gel (10 µm) (Such as Nucleosil C18),
- mobile phase: *0.005 M dioctyl sodium sulphosuccinate* in a mixture of 3 volumes of *glacial acetic acid,* 87 volumes of *water* and 110 volumes of *methanol,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b) and test solution (a). In the chromatogram obtained with the test solution (a), the area of any peak corresponding to 5-aminoimidazole-4-carboxamide hydrochloride is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.6 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light.

Test solution. Dissolve an accurately weighed quantity of powder containing about 0.2 g of Dacarbazine in 50.0 ml of *0.25 M acetic acid.*

Reference solution. A 0.004 per cent w/v solution of *2-azahypoxanthine IPRS* in *0.25 M acetic acid.*

Chromatographic system

- a stainless steel column 20 cm x 4.0 mm, packed with end-capped octadecylsilyl silica gel (10 µm) (Such as Nucleosil C18),
- mobile phase: *0.005 M dioctyl sodium sulphosuccinate* in a mixture of 1.5 volumes of *glacial acetic acid* and 98.5 volumes of *water,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

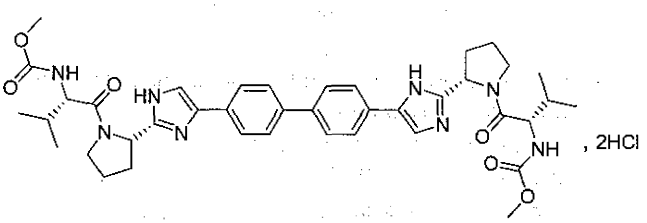
Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and not more than one such peak has an area more than 0.5 times the principal peak in the chromatogram obtained with reference solution (0.5 per cent), and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (3 per cent).

Bacterial endotoxin (2.2.3). Not more than 0.52 Endotoxin Unit per mg of dacarbazine.

Assay. Weigh a quantity of the mixed contents of 10 containers containing 80 mg of Dacarbazine and dissolved in sufficient *0.1 M hydrochloric acid* to produce 100.0 ml; dilute 1.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at 323 nm (2.4.7). Calculate the content of $C_6H_{10}N_6O$ taking 1090 as the specific absorbance at 323 nm.

Storage. Store protected from light and moisture, at a temperature between 2° to 8°.

Daclatasvir Dihydrochloride



$C_{40}H_{50}N_8O_6,2HCl$ Mol. Wt. 811.8

Daclatasvir Dihydrochloride is Methyl((1*S*)-1-(((2*S*)-2-(5-(4'-(2-((2*S*)-1-((2S)-2-((methoxycarbonyl)amino)-3-methylbutanoyl)-2-pyrrolidinyl)-1*H*-imidazol-5-yl)-4-biphenylyl)-1*H*-imidazol-2-yl)-1-pyrrolidinyl) carbonyl)-2-methylpropyl) carbamate dihydrochloride.

Daclatasvir Dihydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent, of $C_{40}H_{50}N_8O_6,HCl$ calculated on the anhydrous and solvent free basis.

Category. Antiviral.

Description. A White to yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *daclatasvir dihydrochloride IPRS* or with the reference spectrum of daclatasvir dihydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). – 102° to – 92°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of mobile phase A and 20 volumes of mobile phase B.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the same solvent.

Reference solution. A 0.00005 per cent w/v solution of *daclatasvir dihydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group bonded to porous silica (5µm) (Such as Kromasil Eternity XT-5),
- column temperature: 45°,
- mobile phase A: a buffer solution prepared by dissolving 3.5 g of *sodium dihydrogen phosphate* in 1000 ml of 0.1 per cent v/v solution of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid.*

  B: *acetonitrile,*
- a gradient program using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
25	55	45
30	55	45
35	85	15
40	85	15

Retention time of the daclatasvir dihydrochloride peak is about 16 minutes.

Inject the reference solution. The test is not valid unless, the column efficiency is not less than 2000 theoretical plates the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than ten times the area of the principal peak in the chromatogram with reference solution (1.0 per cent).

Enantiomeric purity. Not more than 0.15 per cent of D, D daclatasvir dihydrochloride.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of substance under examination in 50.0 ml of mobile phase.

Reference solution (a). A 0.0064 per cent w/v solution of *DD daclatasvir dihydrochloride (Methyl((1R)-1-((2R)-2-(5-(4'-2-((2R)-1-((2R)-2-((methoxy carbonyl)amino)-3-methyl-butanoyl)-2-pyrrolidinyl)-1H-imidazol-5-yl)-4-biphenylyl)-1H-imidazol-2-yl-1-pyrrolidinyl)carbonyl)-2-methylpropyl carbamate dihydrochloride) IPRS* in the mobile phase.

Reference solution (b). Dissolve 50 mg of *daclatasvir dihydrochloride IPRS* in 100.0 ml of mobile phase containing 3.0 ml of reference solution (a).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with cellulose tris-(3,5-dichlorophenyl carbamate bonded to porous silica (3µm) (Such as Chiralpak IC-3),
- column temperature: 40°,
- mobile phase: 30 volumes of a buffer solution prepared by dissolving 1.58 g of *ammonium bicarbonate* in 1000 ml of *water* and 70 volumes of *acetonitrile.*
- flow rate: 1 ml per minute.
- spectrophotometer set at 320 nm,
- injection volume:10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to daclatasvir dihydrochloride and DD-daclatasvir dihydrochloride is not less than 2.0. The relative retention time of DD-daclatasvir dihydrochloride with respect to daclatasvir is about 1.57.

Inject the test solution and calculate the content of DD daclatasvir dihydrochloride in daclatasvir dihydrochloride by area normalization.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Chloride content. Not less than 8.0 per cent and not more than 10.0 per cent.

Dissolve about 0.1g of the substance under examination in a mixture of 50.0 ml of *methanol*, 5.0 ml of *glacial acetic acid* and 5.0 ml of *water*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of chloride.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.1 g in *methanol*.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the sample under examination in 20.0 ml of *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with *methanol*.

Reference solution. A 0.01 per cent w/v solution of *daclatasvir dihydrochloride IPRS* in *methanol.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5µm) (Such as Zorbax Rx, C8),
- column temperature: 35°,
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water* with 10.0 ml of *formic acid* and 5.0 ml of *trimethylamine*, adjusted to pH 2.5 with *formic acid* and 30 volumes of *acetonitrile,*
- spectrophotometer set at 300 nm,
- flow rate: 1.2 ml per minute,
- injection volume: 10 µl

Retention time of the principal peak is about 4 minutes.

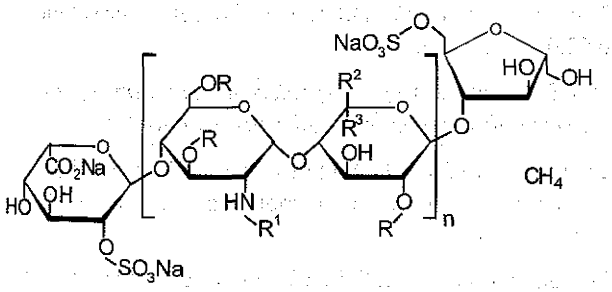
Inject the reference solution. The test is not valid unless, the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{40}H_{50}N_8O_6,2HCl$.

Storage. Store at a temperature between 15° to 30°.

Dalteparin Sodium



n = 3 to 20, R = H or SO_3Na, R^1 = SO_3Na or CO-CH_3
R^2 = H and R^3 = CO_2Na or R^2 = CO_2Na and R^3 = H

Dalteparin Sodium is the sodium salt of a low-molecular-mass heparin that is obtained by nitrous acid depolymerisation of heparin from porcine intestinal mucosa. The majority of the components have a 2-*O*-sulfo-α-L-idopyranosuronic acid structure at the non-reducing end and a 6-*O*-sulfo-2, 5-anhydro-D-mannitol structure at the reducing end of their chain.

Dalteparin Sodium complies with the monograph Enoxaparin Sodium with the modifications and additional requirements below.

The mass-average relative molecular mass range is not less than 5600 and not more than 6400, with a characteristic value of about 6000.

The degree of sulfatation is 2.0 to 2.5 per disaccharide unit.

The potency is not less than 110 IU and not more than 210 IU of anti-factor Xa activity per milligram, calculated with reference to the dried substance. The anti- factor IIa activity is not less than 35 IU per mg and not more than 100 IU per mg, calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.9 and 3.2.

Production

Dalteparin sodium is produced by a validated manufacturing and purification procedure under conditions designed to minimise the presence of N-NO groups.

The manufacturing procedure must have been shown to reduce any contamination by N-NO groups to approved limits using an appropriate, validated quantification method.

Category. Anticogulant; antithrombotic.

Identification

A. Determine by nuclear magnetic resonance spectrometry (2.4.34).

Solvent mixture. 20 ml of *deuterium oxide* and 80 ml of *water.*

Test solution. Dissolve 0.2 g of the substance under examination in 1.0 ml of the solvent mixture.

Reference solution. A 20.0 per cent w/v solution of *dalteparin sodium IPRS* in the solvent mixture.

Using a pulsed (Fourier transform) NMR spectrometer operating at 75 MHz for ^{13}C. Record the spectra at 40°, using cells 5 mm in diameter. . Use deuterated *methanol* as internal reference at δ = 50.0 ppm.

The spectrum obtained is similar to the appropriate specific low-molecular-mass heparin reference standard.

B. Determined by size-exclusion chromatography (2.4.16).

Test solution. Dissolve 20 mg of the substance under examination in 2 ml of the mobile phase.

Reference solution. A 1.0 per cent w/v solution of *heparin low-molecular-mass for calibration IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 7.5 mm, packed with porous silica beads (5 µm) with a fractionation range for proteins of approximately 15000 to 100000,

- mobile phase: a 2.84 per cent w/v solution of *anhydrous sodium sulphate*, adjusted to pH 5.0 with 10 per cent *sulphuric acid*,
- flow rate: 0.5 ml per minute,
- refractive index detector,
- injection volume: 25 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 10000 theoretical plates.

For detection, use a differential refractive index (RI) detector connected in series to a ultraviolet spectrophotometer (UV) set at 234 nm such that the UV monitor is connected to the column outlet, and the RI detector to the UV-monitor outlet.

The normalisation factor used to calculate the relative molecular mass from the RI/UV ratio is obtained as follows: calculate the total area under the UV_{234} (ΣUV_{234}) and the RI (ΣRI) curves by numerical integration over the range of interest (i.e. excluding salt and solvent peaks at the end of the chromatogram). Calculate the ratio r using the following expression.

$$\frac{\sum RI}{\sum UV_{234}}$$

Calculate the factor *f* using the following expression:

$$\frac{M_{na}}{r}$$

where, M_{na} = assigned number-average relative molecular mass of the *heparin low-molecular-mass for calibration IPRS* found in the leaflet supplied with the RS.

Provided the UV_{234} and the RI responses are aligned, the relative molecular mass *M* at any point is calculated using the following expression.

$$f\frac{RI}{UV_{234}}$$

The resulting table of retention times and relative molecular masses may be used to derive a calibration for the chromatographic system by fitting a suitable mathematical relationship to the data. A polynomial of the 3rd degree is recommended. *(It must be stressed that the extrapolation of this fitted calibration curve to higher molecular masses is not valid).*

Inject 25 µl of the test solution and record the chromatogram for a period of time, ensuring complete elution of sample and solvent peaks.

The mass-average relative molecular mass is defined by the following expression.

$$\frac{\sum(RI_i M_i)}{\sum RI_i}$$

where, RI_i = mass of substance eluting in the fraction *i*;
M_i = relative molecular mass corresponding to fraction *i*.

The mass-average relative molecular mass ranges between 5600 and 6400. The mass percentage of chains lower than 3000 is not more than 13.0 per cent. The mass percentage of chains higher than 8000 ranges between 15.0 per cent and 25.0 per cent.

Tests

Appearance of solution. A 10.0 per cent w/v solution is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Nitrite. Not more than 5 ppm.

Determine by liquid chromatography (2.4.14).

NOTE — Rinse all volumetric flasks at least three times with water before the preparation of the solutions.

Test solution. Dissolve 80 mg of the substance under examination in *water* and dilute to 10.0 ml with *water*. Allow to stand for at least 30 minutes.

Reference solution (a). Dissolve 60 mg of *sodium nitrite* in *water* and dilute to 1000.0 ml with *water*.

(For the preparation of reference solution (b), use a pipette previously rinsed with reference solution (a)).

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 50.0 ml with *water*.

NOTE — Before preparing reference solution (c), (d) and (e), rinse all pipettes with reference solution (b).

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 100.0 ml *with water (*corresponding to 1 ppm of nitrite in the test sample).

Reference solution (d). Dilute 3.0 ml of reference solution (b) to 100.0 ml with *water* (corresponding to 3 ppm of nitrite in the test sample).

Reference solution (e). Dilute 5.0 ml of reference solution (b) to 100.0 ml with *water* (corresponding to 5 ppm of nitrite in the test sample).

Chromatographic system

- a stainless steel column 12.5 cm x 4.3 mm, packed with strong anion-exchange resin,
- mobile phase: a solution consisting of 13.61 g of *sodium acetate* dissolved in *water*, adjusted to pH 4.3 with *phosphoric acid* and diluted to 1000 ml with *water*,

- flow rate: 1 ml per minute,
- conductivity detector as an appropriate electrochemical device with the following characteristics and settings: a suitable working electrode, a detector potential of + 1.00 V versus Ag/AgCl reference electrode and a detector sensitivity of 0.1 µA full scale,
- injection volume: 100 µl.

Inject reference solution (d). The retention time for nitrite is 3.3 to 4.0 minutes. The test is not valid unless the column efficiency is not less than 7000 theoretical plates for the nitrite peak. (Dalteparin sodium will block the binding sites of the stationary phase, which will cause shorter retention times and lower separation efficiency for the analyte; the initial performance of the column may be partially restored using a 5.8 per cent w/v solution of *sodium chloride* at a flow rate of 1.0 ml per minute for 1 hour; after regeneration the column is rinsed with 200 ml to 400 ml of *water).* The tailing factor for the peak due to nitrite is not more than 3.0 and the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject reference solution (c) and (e). The test is not valid unless the correlation factor for a linear relationship between concentration and response for reference solution (c), (d) and (e) is not less than 0.995 and the signal-to-noise ratio for reference solution (c) is not less than 5.

Inject the test solution. Calculate the content of nitrite from the peak areas in the chromatogram obtained with reference solution (c), (d) and (e).

Boron. Not more than 1 ppm.

Determine by inductively coupled plasma atomic emission spectroscopy (2.4.3).

Boron is determined by measurement of the emission from inductively coupled plasma (ICP) at a wavelength specific to boron. The emission line at 249.733 nm is used. Use an appropriate apparatus, whose settings have been optimised as directed by the manufacturer.

Test solution. Dissolve 0.25 g of the substance under examination in about 2 ml of *water,* add 100 µl of *nitric acid* and dilute to 10.0 ml with 1.0 per cent v/v solution of *nitric acid.*

Reference solution (a). A 1.0 per cent v/v solution of *nitric acid* in *water* (blank).

Reference solution (b). A 11.4 µg per ml solution of *boric acid* in a 1 per cent v/v solution of *nitric acid* in *water* (STD_{cal}).

Reference solution (c). Dissolve 0.25 g of *dalteparin sodium IPRS* with no detectable boron in about 2 ml of *water,* add 100 µl of *nitric acid,* and dilute to 10.0 ml with1.0 per cent v/v solution of nitric acid (STD_0).

Reference solution (d). Dissolve 0.25 g of a reference dalteparin sodium with no boron detected in about 2 ml of a 1 per cent v/v solution of *nitric acid* in *water,* add 10 µl of a 5.7 mg per ml solution of *boric acid* and dilute to 10.0 ml with the same solvent (STD_1). This solution contains 1 µg per ml of boron.

Calculate the content of boron in the substance under examination, using the following correction factor:

$$f = \frac{(STD_1 - STD_0) \times 2}{(STD_{cal} - \text{blank})}$$

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° over *diphosphorus pentoxide* at a pressure not exceeding 670 Pa for 3 hours.

Storage. Store protected from light and moisture and below 40° temperature.

Dalteparin Sodium Injection

Dalteparin Sodium Injection is a sterile solution of Dalteparin Sodium in a suitable diluent.

The estimated activity of anti-factor Xa is not less than 90 per cent and not more than 110 per cent of the stated activity.

Production

The final product is produced by methods of manufacturing designed to ensure that substances lowering blood pressure are not introduced and to ensure freedom from contamination by over-sulphated glycosaminoglycans.

Usual strengths. 2000 IU per 0.2 ml; 2500 IU per 0.2 ml; 5000 IU per 0.2 ml; 7500 IU per 0.2 ml.

Identification

A. Determine by size-exclusion chromatography (2.4.16) using the following solutions in the mobile phase.

Test solution. Dilute the injection to contain 1600 units of anti-factor Xa per ml.

Reference solution. A 1.0 per cent w/v solution of *heparin low-molecular-mass for calibration IPRS.*

Chromatographic system

- a stainless steel column 30 cm x 7.5 mm, packed with appropriate porous silica beads (5 µm) with a fractionation range for proteins of approximately 15 000 to 100 000 (Such as Waters Protein-Pak and Toso Hass TSK G2000SW),

- mobile phase: a 2.84 per cent w/v solution of *anhydrous sodium sulphate* adjusted to pH 5.0 with *dilute sulphuric acid*,
- flow rate: 0.5 ml per minute,
- a differential refractometer (RI) detector connected in series to an ultraviolet spectrophotometer (UV) set at 234 nm such that the UV monitor is connected to the column outlet, and the RI detector to the UV-monitor outlet. It is necessary to measure the time lapse between the 2 detectors accurately so that their chromatograms can be aligned correctly. The retention times used in the calibration must be those from the RI detector,
- injection volume: 25 µl.

The normalisation factor used to calculate the relative molecular mass from the RI/UV ratio is obtained as follows. Calculate the total area under the UV_{234} (SUV_{234}) and the RI (SRI) curves by numerical integration over the range of interest (i.e. excluding salt and solvent peaks at the end of the chromatogram). Calculate the ratio r using the following expression.

$$\frac{\sum RI}{\sum UV_{234}}$$

Calculate the factor *f* using the following expression.

$$\frac{M_{na}}{r}$$

where, M_{na} = assigned number-average relative molecular mass of the *heparin low-molecular-mass for calibration IPRS* found in the leaflet supplied with the RS.

Provided the UV_{234} and the RI responses are aligned, the relative molecular mass *M* at any point is calculated using the following expression.

$$f\frac{RI}{UV_{234}}$$

The resulting table of retention times and relative molecular masses may be used to derive a calibration curve for the chromatographic system by fitting a suitable mathematical relationship to the data. A polynomial of the 3rd degree is recommended. *(It must be stressed that the extrapolation of this fitted calibration curve to higher molecular masses is not valid).*

Inject the reference solution. The test is not valid unless the column efficiency is not less than 20000 theoretical plates.

Inject the test solution.

The mass-average relative molecular mass is defined by the following expression.

$$\frac{\sum(RI_iM_i)}{\sum RI_i}$$

Where, RI_i = mass of substance eluting in the fraction *i*;
M_i = relative molecular mass corresponding to fraction *i*.

The mass-average relative molecular mass ranges between 5600 and 6400. The mass percentage of chains lower than 3000 is not more than 13.0 per cent. The mass percentage of chains higher than 8000 ranges between 15.0 per cent and 25.0 per cent.

B. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, is not less than 1.9 and not more than 3.2.

C. It gives reaction (A) of sodium salts (2.3.1).

Tests

Appearance of solution The solution is clear (2.4.1) and not more intensely coloured than reference solutionYS3 (2.4.1).

pH (2.4.24). 5.0 to 7.5,

Bacterial endotoxins (2.2.3). Not more than 0.01 Endotoxin Units per IU of anti-Xa activity.

Other tests. Comply with the tests stated under Parenteral Preparation (Injections).

Assay

Anti-factor Xa activity. Not less than 90 and not more than 110 Anti-Factor Xa IU per mg.

Acetic acid solution. Transfer 42 ml of *glacial acetic acid* to a 100-ml volumetric flask, dilute with *water* to volume, and mix.

Polyethylene glycol 6000 buffer pH 7.4. Dissolve 6.08 g of *tris(hydroxymethyl)aminomethane* and 8.77 g of *sodium chloride* in 500 ml of *water.* Add 1.0 g of *polyethylene glycol 6000*, adjusted to pH 7.4 with *hydrochloric acid* and dilute with *water* to 1000 ml.

Buffer pH 7.4. Dissolve 6.08 g of *tris(hydroxymethyl) aminomethane* and 8.77 g of *sodium chloride* in 500 ml of *water.* Adjusted to pH 7.4 with *hydrochloric acid* and dilute to 1000 ml with *water.*

Buffer pH 8.4. Dissolve 3.03 g of *tris(hydroxymethyl) aminomethane*, 5.12 g of *sodium chloride* and 1.40 g of *edetate*

sodium in 250 ml of *water*. Adjusted to pH 8.4 with *hydrochloric acid* and dilute to 500 ml with *water*.

Human antithrombin III solution. Reconstitute a vial of antithrombin III in *water* to obtain a solution containing 5 Antithrombin III Units per ml. Dilute the solution with *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution having a concentration of 1.0 Antithrombin III Unit per ml.

Factor Xa solution. Reconstitute a weighed quantity of bovine factor X*a* in *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution that gives an increase in absorbance value at 405 nm of not more than 0.20 absorbance units per minute when assayed as described below but using as an appropriate volume (V, in µl) of Buffer pH 7.4 instead of *V* µl of the dalteparin solution.

Chromogenic substrate solution. Prepare a solution of a suitable chromogenic substrate for amidolytic test for factor X*a* in water to obtain a concentration of about 3 mM. Dilute with buffer pH 8.4 to obtain a solution having a concentration of 0.5 mM.

Reference solutions. Dilute low molecular mass heparins with Buffer pH 7.4 to obtain four dilutions in the concentration range between 0.025 and 0.2 USP Anti-Factor X*a* IU per ml.

Test solutions. Proceed as directed for reference solutions to obtain concentrations of Dalteparin Sodium similar to those obtained for the reference solutions.

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the test solutions; and S1, S2, S3, and S4 each in duplicate for the dilutions of the reference solutions. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, V, (20 to 50 µl) of *Human antithrombin III solution* and an equal volume, V, of either the blank, buffer pH 7.4, or an appropriate dilution of the test solutions and reference solutions. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 minute. Add to each tube volume 2V (40 to100 µl) of *Factor* Xa solution, and incubate for 1.0 minute. Add 5V (100 to 250 µl) volume of *chromogenic substrate solution*. Stop the reaction after 4.0 minutes with 5V (100 to 375 µl) volume of *acetic acid solution*. Measure the absorbance of each solution at 405 nm against blank B1.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and reference solutions, and calculate the potency of the dalteparin sodium in IU of anti-factor Xa activity per ml using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the anti-factor X*a* activity of Dalteparin Sodium per mg, calculated on the dried basis.

Anti-factor IIa activity

Reference solutions. Dilute low molecular mass heparins with Buffer pH 7.4 to obtain four dilutions in the concentration range between 0.015 and 0.075 USP Anti-Factor II_a activity per ml.

Test solutions. Proceed as directed for reference solutions to obtain concentrations of Dalteparin Sodium similar to those obtained for the reference solutions.

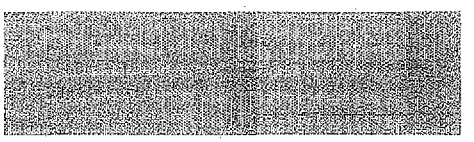
Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the test solutions; and S1, S2, S3, and S4 each in duplicate for the dilutions of the reference solutions. *[NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.]* To each tube add the same volume, V, (20 to 50 µl) of *Human antithrombin III solution* and an equal volume, V, of either the blank, buffer pH 7.4, or an appropriate dilution of the test solutions and reference solutions. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 minute. Add to each tube volume 2V (40 to100 µl) of *Factor* Xa solution, and incubate for 1 minute. Add 5V (100 to 250 µl) volume of *chromogenic substrate solution*. Stop the reaction after 4.0 minutes with 5V (100 to 375 µl) volume of *acetic acid solution*. Measure the absorbance of each solution at 405 nm against blank B1.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and reference solutions, and calculate the potency of the dalteparin sodium in IU of anti-factor Xa activity per ml using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the anti-factor X*a* activity of Dalteparin Sodium per mg, calculated on the dried basis.

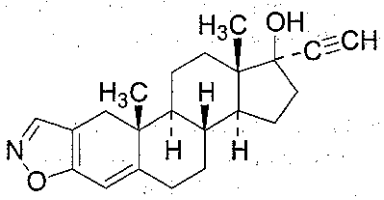
Anti-factor X*a* to anti-factor II*a* ratio. The ratio of the numerical value of the anti-factor X*a* activity in Anti-Factor X*a* IU per mg to the numerical value of the anti-factor II*a* activity in Anti-Factor II*a* IU per mg, as determined by the Assay (anti-factor Xa activity) and the Anti-factor IIa activity, respectively, is not less than 1.9 and not more than 3.2.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states (a) the number of International Units of anti-factor Xa activity per miligram; (b) the number of International Units of anti-factor IIa activity per miligram; (c) the mass-average molecular mass and the percentage of molecules within defined molecular mass ranges; (d) where applicable, that the contents are the sodium salt.



Danazol



$C_{22}H_{27}NO_2$ Mol. Wt. 337.5

Danazol is 17α-pregna-2,4-diene-20-yno[2,3-*d*]isoxazol-17-ol.

Danazol contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{22}H_{27}NO_2$, calculated on the dried basis.

Category. Antigonadotrophin.

Description. A white to pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *danazol IPRS* or with the reference spectrum of danazol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 285 nm.

Tests

Specific optical rotation (2.4.22). +21.0° to +27.0°, determined in a 1.0 per cent w/v solution in *chloroform*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 9 volumes of *chloroform* and 1 volume of *methanol*.

Mobile phase. A mixture of 70 volumes of *cyclohexane* and 30 volumes of *ethyl acetate*.

Test solution. Dissolve 0.5 g of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution (a). Dissolve 50 mg of *danazol IPRS* in 100.0 ml of the solvent mixture.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 20.0 ml with the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in warm air and examine under ultraviolet light at 254 nm. Expose the plate to the vapour of iodine for 5 minutes and examine the plate again. By both methods of visualisation, any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 2.7 kPa.

Assay. Weigh 0.1 g, previously dried, dissolve in 50 ml of *ethanol (95 per cent)*, swirling until dissolved, and dilute to 100.0 ml with *ethanol (95 per cent)*. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7). Calculate the content of $C_{22}H_{27}NO_2$ from the absorbance obtained by repeating the procedure using a solution containing 0.002 per cent w/v of *danazol IPRS* in place of the substance under examination.

Storage. Store protected from light.

Danazol Capsules

Danazol Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of danazol, $C_{22}H_{27}NO_2$.

Usual strengths. 50 mg; 100 mg; 200 mg.

Identification

A. Extract the contents of the capsules containing about 50 mg of Danazol with 50 ml of *chloroform*, filter and evaporate the filtrate to dryness on a water-bath in a stream of nitrogen. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *danazol IPRS* or with the reference spectrum of danazol.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 0.75 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary with dissolution medium at 286 nm (2.4.7). Calculate the content of danazol, $C_{22}H_{27}NO_2$, in the medium from the absorbance obtained from a solution of known concentration of *danazol IPRS*, prepared by dissolving in minimum quantity of *isopropyl alcohol* and diluted with dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{27}NO_2$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the mixed contents of 20 capsules containing 100 mg of Danazol in 50 ml of the mobile phase for 10 minutes and dilute to 100.0 ml with the mobile phase and filter. Dilute 5.0 ml of the filtrate to 25.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *danazol IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile*, 30 volumes of *methanol* and 30 volumes of *water*,
- flow rate: 1.5 ml per minute.
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

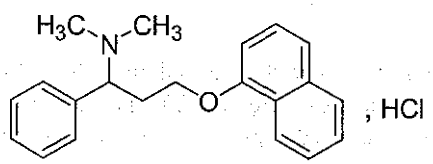
Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{27}NO_2$ in the capsules.

Storage. Store protected from light.

Dapoxetine Hydrochloride

Dapoxetine Hydrochloride monohydrate



$C_{21}H_{23}NO,HCl$ Mol wt. 341.9

Dapoxetine Hydrochloride is (*RS*)-*N*,*N*-Dimethyl-3-(1-naphthyloxy)-1-phenylpropylamine hydrochloride.

Dapoxetine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{23}NO,HCl$, calculated on the dried basis.

Category. Selective serotonin re-uptake inhibitor (SSRI).

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dapoxetine hydrochloride IPRS* or with the reference spectrum of dapoxetine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution. A 0.0004 per cent w/v solution of *dapoxetine hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of 0.1 per cent v/v solution of *trifluoroacetic acid* in *water* and 60 volumes 0.1 per cent v/v solution *trifluoroacetic acid* in *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0.

Inject the test solution, the area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Enantiomeric purity. D-isomer is 48.5 to 51.5 per cent and *L*- isomer is 48.5 to 51.5 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with mobile phase.

Reference solution. A 0.05 per cent w/v solution of *dapoxetine hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, (Such as chiralcel OJ-RH) (5 µm)
- mobile phase: a mixture of 80 volumes of *methanol*, 20 volumes of *ethanol* and 0.1 volumes of *triethylamine*,
- flow rate: 1ml per minute,
- spectrophotometer set at 290 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of *D*-isomer and *L*-isomer is not more than 2.0

and the resolution between D-isomer and L-isomer is not less than 1.5.

Inject the reference solution and the test solution.

Calculate the content of D-isomer and L-isomer.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in mobile phase and dilute to 100.0 ml with mobile phase.

Reference solution. A 0.025 per cent w/v solution of *dapoxetine hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of 0.1 per cent *trifluoroacetic acid* in *water* and 60 volumes 0.1 per cent *trifluoroacetic acid* in *acetonitrile*.
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{23}NO$,HCl.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Dapoxetine Tablets

Dapoxetine Hydrochloride Tablets.

Dapoxetine Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dapoxetine, $C_{21}H_{23}NO$.

Usual strengths. The equivalent of 30 mg; 60 mg of dapoxetine.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *dapoxetine hydrochloride IPRS* in the dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 5.0 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, add 3 ml of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl,
- run time: about 10 minutes.

Inject the reference solution and the test solution.

Q. Not less than 70.0 per cent of the stated amount of $C_{21}H_{23}NO$.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Prepared by dissolving 6.8 g of potassium dihydrogen orthophosphate in 1000 ml of *water*, add 3 ml of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid*.

Solvent mixture. A mixture of equal volumes of buffer and *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 30 mg of dapoxetine add 50 ml of solvent mixture, sonicate for 5 minutes, dilute to 100.0 ml with the solvent mixture. Centrifuge the solution if required.

Reference solution. Dissolve 34 mg of *dapoxetine hydrochloride IPRS* in 50 ml of solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3 µm),

- mobile phase: A. 80 volumes of buffer solution and 20 volumes of *acetonitrile*,
  B. a mixture of 30 volumes of buffer solution and 70 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
8	50	50
14	50	50
15	70	30
20	70	30

Inject the reference solution. The test is not valid unless the column efficiency in not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution, the area of any secondary peak is not more than 1.0 per cent and the sum of area of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 30 mg of Dapoxetine, and transfer to a 100-ml volumetric flask. Add about 50 ml of mobile phase and disperse with the aid of ultrasound for 20 minutes, cool and dilute to volume with mobile phase, filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.033 per cent w/v solution of *dapoxetine hydrochloride IPRS* in mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Use the chromatographic condition as described under Dissolution.

Inject the reference solution. The test is not valid unless the column efficiency in not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

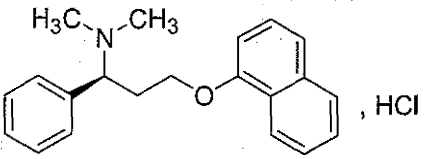
Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{23}NO$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of dapoxetine.

S-Dapoxetine Hydrochloride



$C_{21}H_{23}NO,HCl$ Mol wt. 341.9

S-Dapoxetine Hydrochloride is (*S*)-*N*,*N*-Dimethyl-3-(1-naphthyloxy)-1-phenylpropylamine hydrochloride.

S-Dapoxetine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{23}NO,HCl$, calculated on the anhydrous basis.

Category. Selective serotonin re-uptake inhibitor (SSRI).

Description. A white to off white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *S-dapoxetine hydrochloride IPRS* or with the reference spectrum of *S*-dapoxetine hydrochloride.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. A solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in to a 1000 ml *water*, add 3 ml of *triethylamine* and adjusted to pH 3.0 with *orthophosphoric acid*.

Solvent mixture. Equal volumes of buffer solution and *acetonitrile*.

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture, sonicate to dissolve and dilute to 10.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *S-dapoxetine hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 2.1 mm, packed with octadecylsilane bonded to porous silica (1.7 µm) (Such as Acquity UPLC R BEH),
- column temperature: 50°,
- sample temperature: 5°,
- mobile phase: A. a mixture of 80 volumes of buffer solution and 20 volumes of *acetonitrile*,
  B. a mixture of 30 volumes of buffer solution and 70 volumes of *acetonitrile*,

- flow rate: 0.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 1 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
15	70	30
20	50	50
22	50	50
22.1	85	15
24	85	15

Name	Relative retention time
1-Fluoronephtanlene	2.34
Dapoxetine HCl	1.0

Inject the reference solution. The test is not valid unless the column efficiency is not less than 10000 theoretical plates, tailing factor is not more than 3.0.

Inject the test solution, the area of any secondary peak is not more than 0.2 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Enantiomeric purity. R-isomer is not more than 1 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5.0 mg of the substance under examination in *ethanol*, sonicate to dissolve and dilute to 10.0 ml with the same solvent.

Reference solution. A 0.05 per cent w/v solution of *S-dapoxetine hydrochloride IPRS* in with the *ethanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, (Such as Chiralpak AD-H),
- mobile phase: a mixture of 90 volumes of *n-hexane*, 10 volumes of *isopropyl alcohol*, 0.2 volume of *diethylamine* and 0.2 volume of *trifluoroacetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

The retention time of *S*-dapoxetine and *R*-dapoxetine is about 9.8 and 8.8 minutes respectively.

Inject the reference solution. The test is not valid unless resolution between *R*-isomer and *S*-isomer is not less than 1.5.

Inject the test solution. Calculate the content of *R*-isomer by area normalization method.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Dissolve 0.25g in a 50.0 ml of a mixture of 1 per cent w/v solution of *mercuric acetate* in *anhydrous acetic acid*. Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03419 g of $C_{21}H_{23}NO,HCl$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

S-Dapoxetine Tablets

S-Dapoxetine Hydrochloride Tablets

S-Dapoxetine Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of *S*-dapoxetine, $C_{21}H_{23}NO$.

Usual strengths. 30 mg; 60 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 15 mg of *S-dapoxetine hydrochloride IPRS* and in 60.0 ml of the dissolution medium and sonicate for 10 minutes and dilute to 100.0 ml with the dissolution medium. Dilute 5.0 ml of the solution to 100.0 ml with same solvent.

Use the chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{23}NO$.

Q. Not less than 70 per cent of the stated amounts of $C_{21}H_{23}NO$.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. A solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml *water*, add 3 ml of *triethylamine* and adjusted to pH 3.0 with *orthophosphoric acid.*

Solvent mixture. A mixture of equal volumes of buffer solution and *acetonitrile.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 30 mg of *S*-Dapoxetine to a100-ml volumetric flask, add 50.0 ml of solvent mixture and sonicate for 5 minutes. Make up the volume with solvent mixture mix and centrifuge.

Reference solution (a). 0.034 per cent w/v solution of *S-dapoxetine hydrochloride IPRS* in solvent mixture.

Reference solution (b). 0.030 per cent w/v solution of *1-flouro napthalene impurity IPRS* in solvent mixture.

Reference solution (c). Dilute 1.0 ml of each reference solution (a) and reference solution (b) to 100.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (Such as Hypersil BDS),
- mobile phase: A. a mixture of 80 volumes of buffer solution and 20 volumes of *acetonitrile*,
  B. a mixture of 30 volumes of buffer solution and 70 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
8	50	50
14	50	50
15	70	30
20	70	30

Name	Relative retention time
1-Fluoronephtanlene	2.67
Dapoxetine hydrochloride	1.0

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 5000 theoretical plates, tailing factor is not more than 2.0 for *S*-dapoxetine and the resolution between *S*-dapoxetine and1-fluoronephthalene is not less than 20.0

Inject the test solution, the area of any secondary peak is not more than 1.0 per cent and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 30 mg of *S*-Dapoxetine to a 100-ml volumetric flask, add 50.0 ml of mobile phase and sonicate for 20 minutes. Make up the volume with mobile phase and centrifuge. Dilute 2.0 ml of the solution to 25.0 ml with the mobile phase.

Reference solution. A 0.0027 per cent w/v solution of *S-dapoxetine hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS),
- mobile phase: a mixture of 650 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml *water,* 3.0 ml of *triethylamine* adjusted to pH 3.0 with *orthophosphoric acid* and 350 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0.

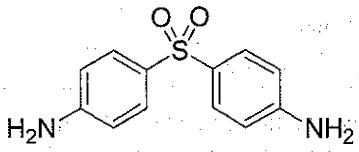
Inject the reference solution and the test solution.

Calculate the contents of $C_{21}H_{23}NO$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of *S*-dapoxetine.

Dapsone



$C_{12}H_{12}N_2O_2S$ Mol. Wt. 248.3

Dapsone is the bis(4-aminophenyl)sulphone.

Dapsone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{12}N_2O_2S$, calculated on the dried basis.

Category. Antileprotic.

Description. A white or creamy-white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dapsone IPRS* or with the reference spectrum of dapsone.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *methanol* shows absorption maxima at 260 nm and 295 nm; absorbance at about 260 nm, about 0.36 and at 295 nm, about 0.6.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. 2 ml of a 0.005 per cent w/v solution in *0.1 M hydrochloric acid* gives the reaction of primary aromatic amines (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 20 volumes of *n-heptane*, 20 volumes of *ethyl acetate*, 6 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of test solution (b) to 10.0 ml with *methanol*.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 10.0 ml with methanol.

Reference solution (c). A 0.1 per cent w/v solution of *dapsone IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of *4-dimethylaminocinnamaldehyde* in a mixture of 99 volumes of *ethanol (95 per cent)* and 1 volume of *hydrochloric acid* and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution. A 0.0025 per cent w/v solution of *dapsone IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with porous silica particles (10 µm),
- mobile phase: a mixture of 10 volumes of *isopropyl alcohol*, 10 volumes of *acetonitrile*, 10 volumes of *ethyl acetate* and 70 volumes of *hexane*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak.

Calculate the content of $C_{12}H_{12}N_2O_2S$.

Storage. Store protected from light.

Dapsone Gel

Dapsone Gel contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Dapsone $C_{12}H_{12}N_2O_2S$.

Usual strength. 5 per cent w/w.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.5 to 7.0

Related substances. Determine by liquid chromatography (2.4.14)

Solvent mixture. a mixture of 75 volumes of *methanol* and 25 volumes of *water*.

Test solution. Disperse a quantity of the gel containing about 50 mg of Dapsone in 35 ml of the solvent mixture, vortex to disperse the sample completely. Further ultrasound for 15 minutes, cool and dilute to 50.0 ml with the solvent mixture and filter, discarding the first few ml of filtrate.

Reference solution. A 0.001 per cent w/v solution of *dapsone IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A . 0.1 per cent solution of *triethylamine* in *water* adjusted to pH 3.0 with *orthophosphoric acid* and filter,

  B. a mixture of 7 volumes of *acetonitrile* and 23 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
30	85	15
35	70	30
60	70	30
65	85	15
70	85	15

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Other tests. Comply with the tests stated under Gel.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. a mixture of 75 volumes of *methanol* and 25volumes of *water*.

Test solution. Disperse a quantity of the gel containing about 50 mg of Dapsone in 35 ml of the solvent mixture, vortex to disperse the sample completely, ultrasound for 15 minutes, cool and dilute to 50.0 ml with the solvent mixture and filter. Discarding the first few ml of filtrate. Dilute 5.0 ml to 50.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *dapsone IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase. a mixture of 70 volumes of 0.1 per cent solution of *triethylamine* in *water*, adjusted to pH 3.0 with *orthophosphoric acid* and filter and 30 volumes of mixture of 7 volumes of *acetonitrile* and 23 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{12}H_{12}N_2O_2S$ in the gel.

Storage. Store below 25°, protect from light and moisture.

Dapsone Tablets

Dapsone Tablets contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of dapsone, $C_{12}H_{12}N_2O_2S$.

Usual strengths. 25 mg; 50 mg; 100 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1g of Dapsone with 10 ml of *acetone*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dapsone IPRS* or with the reference spectrum of dapsone.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a 2 per cent w/v solution of *hydrochloric acid*,

Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Transfer a measured volume of the filtrate containing about 0.2 mg of Dapsone to a 25-ml volumetric flask, add 5 ml of *1 M sodium hydroxide*, dilute to volume with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of $C_{12}H_{12}N_2O_2S$ from the absorbance obtained from a solution prepared by adding 5 ml of *1 M sodium hydroxide* to 20 ml of a 2 per cent v/v solution of *hydrochloric acid* containing 0.2 mg of *dapsone IPRS* and adding sufficient *water* to produce 25.0 ml.

Q. Not less than 75 per cent of the stated amount of $C_{12}H_{12}N_2O_2S$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 20 volumes of *n-heptane*, 20 volumes of *ethyl acetate*, 6 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution (a). Shake a quantity of the powdered tablets containing 0.1 g of Dapsone with 10 ml of *methanol* and filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of test solution (b) to 10.0 ml with *methanol*.

Reference solution (b). Dilute 2 ml of reference solution (a) to 10 ml with *methanol*.

Reference solution (c). A 0.1 per cent w/v solution of *dapsone IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of *4-dimethylaminocinnamaldehyde* in a mixture of 99 volumes of *ethanol (95 per cent)* and 1 volume of *hydrochloric acid* and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

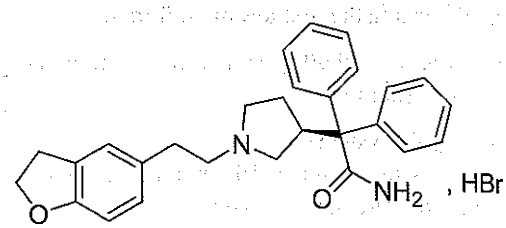
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.25 g of Dapsone and dissolve in a mixture of 15 ml of *water* and 15 ml of *2 M hydrochloric acid*, add 3 g of *potassium bromide*. Cool the solution to about 15° and determine by the nitrite titration (2.3.31). Carry out a blank titration.

1 ml of *0.1 M sodium nitrite* is equivalent to 0.01242 g of $C_{12}H_{12}N_2O_2S$.

Storage. Store protected from light.

Darifenacin Hydrobromide



$C_{28}H_{30}N_2O_2,HBr$ Mol. Wt. 507.5

Darifenacin Hydrobromide is (3S)-1-[2-(2,3-dihydrobenzofuran-5-yl)ethyl]-3-pyrrolidnyl}-2,2-diphenylacetamide hydrobromide.

Darifenacin Hydrobromide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{28}H_{30}N_2O_2,HBr$, calculated on the anhydrous basis.

Category. Antispasmodic.

Description. A white to almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *darifenacin hydrobromide IPRS* or with the reference spectrum of darifenacin hydrobromide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of bromides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 30.0 ml solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). 0.015 per cent w/v solution of *hydrobromic acid* in the solvent mixture.

Reference solution (b). 0.0005 per cent w/v solution of *darifenacin hydrobromide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane chemically bonded to porous silica (5 µm) (Such as Hypersil BDS),
- mobile phase: A. a buffer solution prepared by dissolving 2.76 g of *sodium dihydrogen orthophosphate monohydrate* in 1000 ml of 0.2 per cent v/v solution of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid* and filter,

  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	74	26
2	74	26
12	70	30
25	65	35
40	35	65
41	74	26
50	74	26

Name	Relative retention time	Correction factor
Hydrobromic acid	0.11	—
Darifenacin impurity A[1]	0.23	1.24
Darifenacin impurity B[2]	0.59	1.37
Darifenacin (Retention time: about 16 minutes)	1.0	—
Darifenacin impurity D[3]	1.39	0.79
Darifenacin impurity E[4]	1.57	0.87
Darifenacin impurity F[5]	1.95	1.14
Darifenacin impurity G[6]	2.14	1.18
Darifenacin impurity H[7]	2.25	1.1

[1]dari acetamide compound, [diphenyl [(3S)-pyrrolidin-3-yl]acetamide tartrate],

[2]dari cyano compound, [diphenyl [(3S)-pyrrolidin-3-yl] acetonitrile hydrobromide],

[3]darifenacin oxidised impurity, {2-[1-(2-benzofuran-5-yl)ethyl] pyrrolidin-3-yl}-2,2- diphenylacetamide,

[4] bromo darifenacin impurity, 2{1-[2-(7-bromo-2, 3- dihydrobenzofuran-5-yl)ethyl]-3- pyrrolidinyl)-2,2- diphenylacetamide,

[5]cyano darifenacin impurity, [2-(1-(2-(2,3- dihydrobenzofuran-5-yl) ethyl) pyrrolidin 3-yl)-2,2- diphenylacetonitrile],

[6]darifenacin bromo compound, [5-(2-bromoethy)-2,3-dihydro-benzofuran,

[7]darifenacin dimer impurity [2-(1-(2-(2,3-dihydrobenzofuran-5-yl) ethyl)pyrrolidin-3-yl)-*N*-(2-(2,3-dihydrobenzofuran-5-yl)ethyl)-2,2-diphenylacetamide].

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor for the principal peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to darifenacin impurities A, B,D,E,F,G and H, each of, are not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram with reference solution (b) (1.0 per cent). Ignore any peak due to hydrobromic acid.

Enantiomeric purity. Not more than 1.0 per cent of R-enantiomer.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, (Such as chiralpak IC) (5 µm),
- column temperature: 15°,
- mobile phase: a mixture of 50 volumes of *n-hexane*, 50 volumes of *ethanol* and 0.3 volumes of *diethylamine*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

The relative retention time of R-enantiomer with respect to darifenacin is about 0.8.

Inject the test solution. Calculate the content of the *R*-enantiomer by area normalization.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.1g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of equal volumes of *water* and *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 30.0 ml of solvent mixture and dilute to 50.0 ml

with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *darifenacin hydrobromide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane chemically bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 2.76 g of *sodium dihydrogen orthophosphate monohydrate* in 1000 ml of 0.2 per cent v/v solution of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid*,

B. *acetonitrile,*

- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	74	26
2	74	26
17	68	32
19	25	75
22	25	75
23	74	26
28	74	26

Retention time of the principal peak is about 16 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{28}H_{30}N_2O_2$,HBr.

Storage. Store protected from light and moisture.

Darifenacin Prolonged-release Tablets

Darifenacin Hydrobromide Prolonged-release Tablets

Darifenacin Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Darifenacin Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of darifenacin, $C_{28}H_{30}N_2O_2$.

Usual strengths. 7.5 mg; 15 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powdered tablets containing 30 mg of darifenacin in 30 ml of *acetonitrile* with the aid of ultrasound for 20 minutes, cool, dilute to 50.0 ml with *acetonitrile* and filter. Dilute 5 .0 ml of the solution to 10.0 ml with the *water.*

Reference solution. A 0.00036 per cent w/v solution of *darifenacin hydrobromide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- mobile phase: A. Dissolve 1.38 g of *sodium dihydrogen phosphate monohydrate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and filter,

B. *acetonitrile,*

- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
25	55	45
30	45	55
33	45	55
35	80	20
38	80	20

Name	Relative retention time	Correction factor
Darifenacin	1.0	—
Darifenacin impurity D[1]	1.20	0.76
Darifenacin impurity E[2]	1.32	0.85

[1]darifenacin oxidised impurity, {2-[1-(2-benzofuran-5-yl)ethyl] pyrrolidin-3-yl}-2,2- diphenylacetamide,

'bromo darifenacin impurity, 2{1-[2-(7-bromo-2, 3- dihydrobenzofuran-5-yl)ethyl]-3- pyrrolidinyl)-2,2- diphenylacetamide.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to darifenacin impurity D and E is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent), the area of any other secondary peak is not more than 0. 2 times the area of the principal peak in the chromatogram obtained with the reference solution (0. 2 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and peaks with relative retention time 0.11 with reference to principal peak.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4. 14), as described under Assay, using the following solution.

Test solution. Disperse one intact tablet in 15.0 ml *methanol* with the aid of ultrasound for 10 minutes and dilute to 25.0 ml with the *methanol*, filter. Dilute 2.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution. Dissolve 36 mg of *darifenacin hydrobromide IPRS* in 70 ml of *methanol* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with the mobile phase.

Inject the reference solution and the test solution.

Calculate the content of $C_{28}H_{30}N_2O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 60.0 mg of darifenacin in 70.0 ml of *methanol* with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with the *methanol*, filter. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase.

Reference solution. Dissolve 36 mg of *darifenacin hydrobromide IPRS* in 70 ml of *methanol* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- mobile phase. a mixture of 700 volumes of a buffer solution prepared by dissolving 1.38 g of *sodium dihydrogen phosphate monohydrate* in 1000.0 ml of *water*, adjusted to pH 2.5 with *orthophosphoric* and 300 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

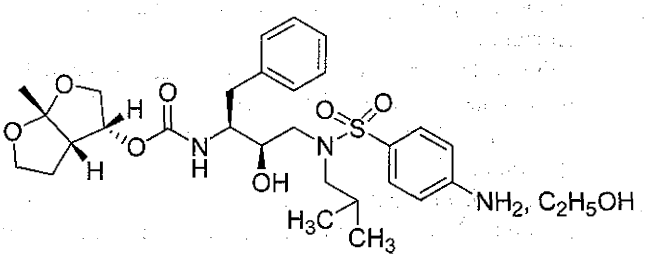
Inject the reference solution and the test solution.

Calculate the content of $C_{28}H_{30}N_2O_2$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of darifenacin.

Darunavir Ethanolate



$C_{27}H_{37}N_3O_7S.C_2H_5OH$ Mol. Wt. 593.7

Darunavir Ethanolate is [(1*S*,2*R*)-3-[[4-aminophenyl) sulfonyl] (2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid (3*R*, 3a*S*, 6a*R*)-hexahydrofuro [2,3-b] furan-3-yl ester monoethanolate.

Darunavir Ethanolate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{27}H_{37}N_3O_7S$, calculated on anhydrous and ethanol free basis.

Category. Antiretroviral.

Description. A white to off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *darunavir ethanolate IPRS* or with the reference spectrum of darunavir ethanolate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

NOTE — After opening the sample, carry out ethanol content, water content and assay simultaneously.

Specific optical rotation (2.4.22). +35° to +41°, on anhydrous and ethanol free basis at 20°, determined in a 1.0 per cent w/v solution in *acetone*, at 365 nm.

Ethanol content. Not less than 5.0 per cent and not more than 8.5 per cent, determined by gas chromatography (2.3.13).

Solvent mixture. Dilute 0.4 g of *isopropyl alcohol* to 500 ml with *dimethylformamide*.

Test solution. Dissolve 50 mg of the substance under examination in 5.0 ml of the solvent mixture.

Reference solution. Dilute 1.6 g of *ethanol* in solvent mixture and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with solvent mixture.

Chromatographic system

- a fused silica column 30 m x 0.53 mm, coated with 94 per cent dimethyl polysiloxane and 6 per cent cyanopropylphenyl (film thickness 3 µm),
- temperature:
  column. 45° for 8 minutes, then raised at the rate of 45° per minute to 230°, for 4 minutes,
  inlet port at 200° and detector at 260°
- split ratio: 1:5,
- flame ionization detector,
- flow rate: 3 ml per minute, using nitrogen as the carrier gas,
- injection volume: 1 µl.

Inject the reference solution. The test is not valid unless the resolution between the peak due to ethanol and isopropyl alcohol is not less than 1.2 and the relative standard deviation for the ratio of the peak areas due to ethanol and isopropyl alcohol for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. Calculate the content of ethanol from the ratio of peak area due to ethanol and isopropyl alcohol in the chromatogram obtained with the reference solution and the test solution.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *water* and 50 volumes of *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 25.0 ml with the same solvent.

Reference solution. A 0.0001 per cent w/v solution of *darunavir ethanolate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a buffer solution prepared by dissolving 2.4 g of *sodium perchlorate* in 1000 ml of *water*, adjusted to pH 2.5 with *perchloric acid*,
  B. a mixture of equal volumes of *acetonitrile* and *methanol*,
- a gradient program using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
5	60	40
30	30	70
35	30	70
35.1	60	40
45	60	40

The relative retention time with reference to principal peak (retention time is about 13 minutes) for difurofuranyl impurity is about 1.46.

Inject the reference solution. The test is not valid unless, the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to di-furofuranyl impurity is not more than the area of the principal peak in the chromatogram obtained with reference solution (0.1 per cent), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 7 times the area of the principal peak in the chromatogram with reference solution (0.7 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substance with the following modifcation.

Test Solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with

the same solvent. Further dilute 10.0 ml of the above solution to 20.0 ml with the solvent mixture.

Reference solution. A 0.025 per cent w/v solution of *darunavir ethanolate IPRS* in the solvent mixture.

Mobile phase. A mixture of equal volumes of mobile phase A and mobile phase B.

Retention time of the principal peak is about 5 minutes.

Inject the reference solution. The test is not valid unless, the column efficiency of the principal peak is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak and measure the responses for the principal peak.

Calculate the content of $C_{27}H_{37}N_3O_7S$.

Storage. Preserve in tight, light resistant containers and store at a controlled temperature between 15° to 30°.

Darunavir Tablets

Darunavir Ethanolate Tablets

Darunavir ethanolate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of darunavir, $C_{27}H_{37}N_3O_7S$.

Usual strengths. 600 mg; 800 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution. (2.5.2)

Apparatus No. 2 (Paddle),
Medium: 900 ml of a buffer solution prepared by dissolving 6.9 g of *sodium dihydrogen orthophosphate monohydrate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, add 20 g of *tween 20*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. a mixture of 25 volumes of *water* and 75 volumes of *methanol.*

Test solution. Use the filtrate, dilute if necessary, with the solvent mixture.

Reference solution. A 0.078 per cent w/v solution of *darunavir ethanolate IPRS* in the solvent mixture. Further dilute the solution with the solvent mixture to obtain a solution having a known concentration similar to that of the test solution. Add 4 per cent v/v of dissolution medium before making up the volume with the solvent mixture.

Chromatographic system
- a stainless steel column 5 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 2.8 g of *sodium perchlorate monohydrate* in 1000 ml of *water*, adjusted to pH 2.5 with *perchloric acid,* 25 volumes of *acetonitrile* and 25 volumes of *methanol.*
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{37}N_3O_7S$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{27}H_{37}N_3O_7S$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture A. Equal volumes of *acetonitrile* and *methanol.*

Solvent mixture B. Equal volumes of *acetonitrile* and *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 100 mg of darunavir in the solvent mixture B, sonicate for 20 minutes with intermittent swirling, cool and dilute to 100.0 ml with the solvent mixture B, mix well and filter.

Reference solution. A solution of *darunavir ethanolate IPRS* in the solvent mixture B containing 0.0002 per cent w/v of darunavir.

Chromatographic system
- a stainless steel column 5 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a mixture of 75 volumes of a buffer solution prepared by dissolving 2.8 g of *sodium perchlorate monohydrate* in 1000 ml of *water* and filter, adjusted to pH 2.5 with *perchloric acid* and 25 volumes of solvent mixture A,
  B. a mixture of 25 volumes of a buffer solution prepared by dissolving 2.8 g of *sodium perchlorate* in 1000 ml of *water* and filter, adjusted to pH 2.5 with *perchloric acid* and 75 volumes of solvent mixture A,

- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
7	60	40
17.5	10	90
25	10	90
25.1	60	40
32	60	40

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any other secondary peak is not more than 2.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0. 5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Other tests. Complies with the tests stated under Tablets.

Water (2.3.43).Not more than 8.0 per cent, determined on 0.5g of the powdered tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile.*

Test solution. Weigh and transfer 5 intact tablets into a 500-ml volumetric flask. Add 250 ml of *water* and sonicate for 15 minutes with intermittent swirling, cool and add 200 ml of *acetonitrile,* sonicate for 10 minutes, cool and dilute to volume with *acetonitrile* and shake well for 2-3 minutes to mix and filter. Dilute with the solvent mixture to obtain a solution of the same concentration as that of the reference solution.

Reference solution. A solution of *darunavir ethanolate IPRS* in the solvent mixture containing 0.035 per cent w/v of darunavir.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3 µm)
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 2.8 g of *sodium perchlorate monohydrate* in 1000 ml of *water*, adjusted to pH 2.5 with *perchloric acid,* 25 volumes of *acetonitrile and* 25 volumes of *methanol.*
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

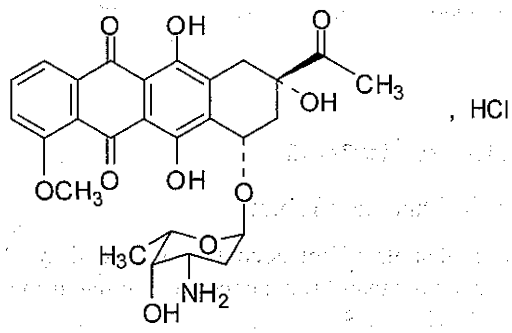
Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{37}N_3O_7S$ in the tablets.

Storage. Store in a cool dry place.

Labelling. The label states the strength in terms of equivalent amount of darunavir.

Daunorubicin Hydrochloride



$C_{27}H_{30}ClNO_{10}$ Mol. Wt. 564.0

Daunorubicin Hydrochloride is (8*S-cis*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride.

Daunorubicin Hydrochloride contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{27}H_{30}ClNO_{10}$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A crystalline, orange-red powder, hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *daunorubicin hydrochloride IPRS* or with the reference spectrum of daunorubicin hydrochloride.

B. Dissolve about 10 mg in 0.5 ml of *nitric acid*, add 0.5 ml of *water* and heat over a flame for 2 minutes. Allow to cool and add 0.5 ml of *silver nitrate solution*; a white precipitate is formed.

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 0.5 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *daunorubicin hydrochloride IPRS* in the mobile phase.

Reference solution (b). Dissolve 10 mg of *doxorubicin hydrochloride IPRS* and 10 mg of *epirubicin hydrochloride IPRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (c). Dissolve 5.0 mg of *daunorubicinone IPRS* and 5.0 mg of *doxorubicin hydrochloride IPRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (d). Dilute 1.0 ml of reference solution (a) to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *acetonitrile* and a solution containing 0.288 per cent w/v of *sodium laurylsulphate* and 0.225 per cent w/v of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5 µl.

The relative retention time with reference to daunorubicin for (8*S*,10*S*)-8-acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicin impurity A) is about 0.4, for doxorubicin (daunorubicin impurity D) is about 0.5, for epirubicin is about 0.6 and for (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-[(1*RS*)-1-hydroxyethyl]-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicin impurity B) is about 0.7.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to doxorubicin hydrochloride and epirubicin hydrochloride is not less than 2.0.

Inject reference solution (b), (c),(d) and the test solution. Run the chromatogram twice the retention time of daunorubicin peak. In the chromatogram obtained with test solution, the area of secondary peak corresponding to daunorubicin impurity A is not more than the area of corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of secondary peak corresponding to daunorubicin impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (d) (1.5 per cent), the area of secondary peak corresponding to daunorubicin impurity D is not more than the area of corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent) and sum of areas of all other secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (2.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Butanol (5.4). Not more than 1.0 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined on 0.1 g.

Daunorubicin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 4.3 Endotoxin Units per mg of daunorubicin hydrochloride.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{27}H_{30}ClNO_{10}$.

Storage. Store protected from light and moisture.

Daunorubicin Injection

Daunorubicin Hydrochloride Injection

Daunorubicin Injection is a sterile material consisting of Daunorubicin Hydrochloride and Mannitol, with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirement for the Appearance of solution and Particulate matter stated under Parenteral Preparations (Injections).

Usual strength. 20 mg.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Daunorubicin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of daunorubicin, $C_{27}H_{29}NO_{10}$.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 4.5 to 6.5, determined on the constituted solution.

Water (2.3.43). Not more than 3.0 per cent, determined on 0.1 g.

Bacterial endotoxins (2.2.3). Not more than 4.3 Endotoxin Units per mg of daunorubicin.

Other tests. Comply with the tests stated under Parenteral Preparations (Powder for Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reconstitute 1 vial with 5 ml of mobile phase and transfer to 200-ml volumetric flask. Wash the vial twice with 5 ml of mobile phase and transfer to the same volumetric flask. Repeat the same procedure for another 9 vials and dilute to volume. Further dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (a). A 0.025 per cent w/v solution of *daunorubicin hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 25 mg of *doxorubicin hydrochloride IPRS* in 100.00 ml of reference solution (a).

Chromatographic system

- a stainless steel column 30 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 62 volumes of *water* and 38 volumes of *acetonitrile,* adjusted to pH 2.2 with *orthophosphoric acid,*
- flow rate 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5 µl.

The relative retention time with reference to *daunorubicin* for *doxorubicin* is about 0.7.

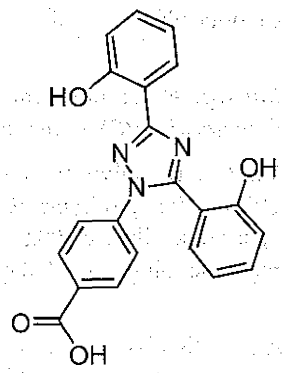
Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to doxorubicin and the daunorubicin is not less than 3.0 in the chromatogram obtain with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtain with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{27}H_{29}NO_{10}$ in the injection.

Storage. Store protected from light.

Deferasirox



$C_{21}H_{15}N_3O_4$ Mol. Wt. 373.4

Deferasirox is 4-[3,5-Bis (2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl]-benzoic acid.

Deferasirox contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{15}N_3O_4$, calculated on the dried basis.

Category. Iron-chelating agent.

Description. A white to slightly yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *deferasirox IPRS* or with the reference spectrum of deferasirox.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

4-Hydrazino benzoic acid. Not more than 0.5 ppm.

Determine by liquid chromatography (2.4.14).

NOTE — Add solvent mixture very slowly with stirring.

Solvent mixture. 10 volumes of *water,* 90 volumes of *acetone* and 2.5 volumes of *orthophosphoric acid.*

Test solution. Dissolve 0.6 g of substance under examination in 2.0 ml of *dimethyl sulphoxide,* with the aid of mechanical shaker. Add slowly 2.0 ml of the solvent mixture with continuous mixing on mechanical shaker. Heat the solution at 45° for 35 minutes, then cool to 2° to 8° and dilute to 5.0 ml with mobile phase A (previously cooled to 2° to 8°). Shake

vigorously with mechanical shaker for about 3 minutes. Centrifuge immediately at 4800 rpm for 5 minutes and filter. Allow the filtrate to stand for 1 hour before injection.

Reference solution (a). Dissolve 6 mg of *4-hydrazino benzoic acid IPRS* in 1 ml of *dimethyl sulphoxide* and dilute to 20.0 ml with *water*. Dilute 1.0 ml of the solution to 10.0 ml with *dimethyl sulfoxide*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with *dimethyl sulphoxide*. Transfer 1.0 ml of the solution to 50-ml volumetric flask, add 4.0 ml of *dimethyl sulphoxide* and 10.0 ml of the solvent mixture. Heat the solution at 45° for 35 minutes, then cool to 2° to 8° and dilute to volume with mobile phase A (previously cooled to 2° to 8°).

Reference solution (c). Transfer 2.0 ml of reference solution (b) to 10-ml volumetric flask, add 1.0 ml of *dimethyl sulphoxide*, 4.0 ml of the solvent mixture and dilute to volume with mobile phase A.

Reference solution (d). Weigh 0.6 g of *deferasirox IPRS* and transfer to 5-ml volumetric flask, add 200 µl of reference solution (a), 2.0 ml of *dimethyl sulphoxide* and shake well to dissolve. Add 2.0 ml of the solvent mixture and mix well using mechanical shaker. Heat the solution at 45° for 35 minutes, then cool to 2° to 8° and dilute to volume with mobile phase A (previously cooled to 2° to 8°). Shake vigorously with mechanical shaker for about 2 minutes. Centrifuge immediately at 4800 rpm for 5 minutes and filter. Allow the filtrate to stand for 1 hour before injection.

Chromatographic system

- a stainless steel column 15 cm × 3 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 10 volumes of *acetonitrile*, 90 volumes of *water* and 0.2 volume of *orthophosphoric acid*,

  B. a mixture of 90 volumes of *acetonitrile*, 10 volumes of *water* and 0.2 volume of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- a gradient programme using the conditions given below,
- spectrophotometer set at 316 nm,
- injection volume: 25 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
2	90	10
8	58	42
8.1	0	100
16	0	100
16.1	90	10
20	90	10

The relative retention time with reference to deferasirox for 4-hydrazino benzoic acid is about 0.5.

Inject reference solution (b), (c) and (d). The test is not valid unless the resolution between the peaks due to 4-hydrazino benzoic acid and deferasirox is not less than 3.0 in the chromatogram obtained with reference solution (d), the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0, the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (b) and the signal to noise ratio is not less than 10 in the chromatogram obtained with reference solution (c).

Inject reference solution (b) and the test solution.

Calculate the content of 4-Hydrazino benzoic acid.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Solvent mixture. 75 volumes of *acetonitrile* and 25 volumes of a solution prepared by dissolving 40 mg of *disodium edetate* in 1000 ml of *water*.

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a). A 0.0025 per cent w/v solution of *deferasirox IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (b). A 0.0005 per cent w/v solution of *deferasirox 1,3-isomer IPRS* in the solvent mixture.

Reference solution (c). Transfer 10 mg of *deferasirox IPRS* in 20-ml volumetric flask, add 10 ml of the solvent mixture, shake well to dissolve, add 1.0 ml of reference solution (b) and dilute to volume with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 20°,
- sample temperature: 5°,
- mobile phase: A. 0.1 per cent v/v solution of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid*,

  B. *acetonitrile*,
- flow rate: 1 ml per minute,
- a gradient programme using the conditions given below,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	55	45
10	55	45
30	50	50
50	20	80
65	20	80
65.1	55	45
75	55	45

Name	Relative retention time	Correction factor
Salicylamide[1]	0.15	0.75
Salicylic acid[2]	0.18	0.79
Cyclised DEF-I compound[3]	0.73	1.49
Deferasirox-1, 2-isomer[4]	0.89	0.76
Deferasirox-1, 3-isomer[5]	0.96	---
Deferasirox (Retention time: about 27 minutes)	1.0	---

[1]2-hydroxybenzamide,

[2] 2-hydroxybenzoic acid,

[3]2-(2-hydroxyphenyl)-4*H* -benzo[e] [1,3]oxazin-4-one; benzoxazinone Analog (DEF-I),

[4]2-[3,5-Bis (2-hydroxyphenyl)-1*H*-1, 2, 4-triazol-1-yl] benzoic acid,

[5]3-[3,5-Bis (2-hydroxyphenyl)-1*H*-1, 2, 4-triazol-1-yl] benzoic acid.

Inject reference solution (a) and (c). The test is not valid unless the resolution between the peaks due to deferasirox and deferasirox-1,3-isomer is not less than 1.3 in the chromatogram obtain with reference solution (c), the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to each of, salicylamide, salicylic acid, cyclized DEF-I compound and deferasirox-1, 2-isomer is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent). The sum of the areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram with reference solution (a) (0.2 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Test solution. Dissolve 25 mg of the substance under examination in 5 ml of *acetonitrile* and 20 ml of the mobile phase with the aid of ultrasound and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve 10 mg of *deferasirox IPRS* in 2 ml of *acetonitrile* and 10 ml of the mobile phase with the aid of ultrasound and dilute to 20.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 50 volumes of 0.1 per cent v/v solution of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{15}N_3O_4$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Deferasirox Tablets

Deferasirox Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of deferasirox, $C_{21}H_{15}N_3O_4$.

Usual strengths. 90 mg; 180 mg; 360 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 0.9 g of *sodium hydroxide* in 900 ml of *water*, adjusted to pH 6.8 with *2 M sodium hydroxide* or *2 M hydrochloric acid* and dilute to 1000 ml with *water*, add 5.0 g of *tween 20* and mix.

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 55 volumes of *water* and 45 volumes of *acetonitrile.*

Test solution. Dilute a suitable volume of the filtrate with the solvent mixture to obtain a solution containing 0.001 per cent w/v of Deferasirox.

Reference solution. Weigh 10 mg of *deferasirox IPRS* in 5 ml of *methanol* with the aid of ultrasound for 5 minutes and dilute to 100.0 ml with the dissolution medium. Dilute a suitable volume of the solution with the solvent mixture to obtain a solution having similar concentration to the test solution.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{15}N_3O_4$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{21}H_{15}N_3O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. A 0.1 per cent v/v solution of *triethylamine*, adjusted to pH 3.0 with *dilute orthophosphoric acid.*

Solvent mixture. 30 volumes of the buffer solution and 70 volumes of *acetonitrile.*

Test solution. Disperse a quantity of powdered tablets containing 50 mg of Deferasirox in 150 ml of the solvent mixture, with the aid of ultrasound for 20 minutes and dilute to 200.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *deferasirox IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of the buffer solution and 55 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 0.9 g of *sodium hydroxide* in 1000 ml of *water*, adjusted to pH 7.4 with *2 M sodium hydroxide* or *2 M hydrochloric acid* and 60 volumes of *acetonitrile.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.3 g of Deferasirox in the solvent mixture, with the aid of ultrasound for about 30 minutes, and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase and filter.

Reference solution. A 0.3 per cent w/v solution of *deferasirox IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 5 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 50°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, add 1 ml of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid*, 40 volumes of *acetonitrile* and 5 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 297 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

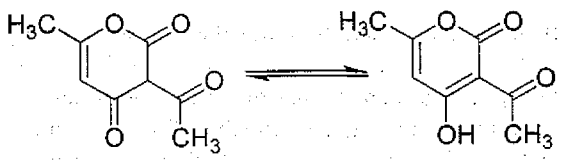
Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{15}N_3O_4$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states that the tablets should be dispersible in water immediately before use, if the tablets are dispersible.

Dehydroacetic Acid



$C_8H_8O_4$ Mol. Wt. 168.1

Dehydroacetic Acid is a tautomeric mixture of 3-acetyl-6-methyl-*2H*-pyran-2,4(*3H*)-dione and 3-acetyl-4-hydroxy-6-methyl-*2H*-pyran-2-one

Dehydroacetic Acid contains not less than 98.0 per cent and not more than 100.5 per cent of $C_8H_8O_4$, calculated on the anhydrous basis.

Category. Pharmaceutical aid (antimicrobial preservative).

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dehydroacetic acid IPRS*.

B. Melts at 109° to 111° (2.4.21).

Tests

Arsenic (2.3.10). Heat gently 3.3 g with 2 ml of *nitric acid* and 0.5 ml of *sulphuric acid* in a long-necked flask until the first reaction has subsided, cool, add carefully and in small portions, 15 ml of *nitric acid* and 6 ml of *sulphuric acid*, taking care to avoid excessive foaming. Continue heating, adding further small portions of *nitric acid*, if necessary, until white fumes are evolved and the solution becomes colourless or almost colourless. Cool, add carefully 10 ml of *water*, evaporate until white fumes are evolved. Repeat the addition of *water* and evaporation until all the *nitric acid* has been removed, cool, dilute to 50 ml with *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

Water (2.3.43). Not more than 1.0 per cent, determined on 2.0 g.

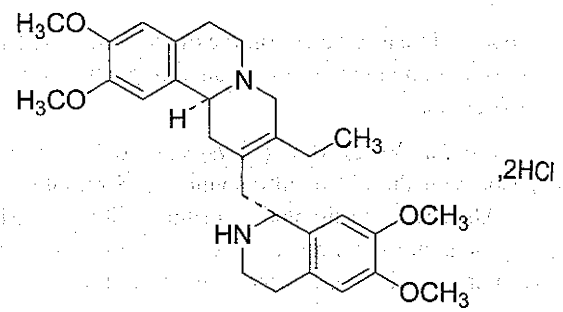
Assay. Dissolve 0.5 g in 75 ml of previously neutralised *ethanol (95 per cent)*, add *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* to a pink end-point that persists for not less than 30 seconds.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01681 g of $C_8H_8O_4$.

Storage. Preserve in well closed containers.

Dehydroemetine Hydrochloride

Dehydroemetine Dihydrochloride



$C_{29}H_{38}N_2O_4,2HCl$ Mol. Wt. 551.6

Dehydroemetine Hydrochloride is 2,3-didehydro-6′,7′,10, 11-tetramethoxyemetan dihydrochloride

Dehydroemetine Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{29}H_{38}N_2O_4,2HCl$, calculated on the dried basis.

Category. Antiamoebic.

Description. A white to yellowish-white, crystalline powder.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at 282 nm, about 0.62.

B. Sprinkle 5 mg on the surface of a 5 per cent w/v solution of *ammonium molybdate* in *sulphuric acid*; a green colour develops.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution YS5 or BYS6 (2.4.1).

pH (2.4.24). 3.5 to 5.0, determined in a 3.0 per cent w/v solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Dissolve 0.4 g in 40 ml of *anhydrous glacial acetic acid* and add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02758 g of $C_{29}H_{38}N_2O_4,2HCl$.

Storage. Store protected from light.

Dehydroemetine Injection

Dehydroemetine Hydrochloride Injection

Dehydroemetine Injection is a sterile solution of Dehydroemetine Hydrochloride in Water for Injections.

Dehydroemetine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dehydroemetine hydrochloride, $C_{29}H_{38}N_2O_4,2HCl$.

Usual strength. 30 mg per ml.

Description. A clear solution.

Identification

A. To a volume containing 30 mg of Dehydroemetine Hydrochloride add 1 ml of *0.05 M iodine*; a yellowish-brown precipitate is produced.

B. To a volume containing 15 mg of Dehydroemetine Hydrochloride add 1 ml of *potassium mercuri-iodide solution*; a white precipitate is produced.

Tests

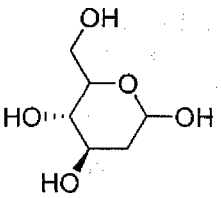
pH (2.4.24). 2.8 to 5.0.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume containing 60 mg of Dehydroemetine Hydrochloride add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with *0.1 M hydrochloric acid*, mix and measure the absorbance of the resulting solution at the maximum at 282 nm (2.4.7). Calculate the content of $C_{29}H_{38}N_2O_4,2HCl$ taking 123 as the specific absorbance at the maximum at 282 nm.

Storage. Store protected from light, in single dose container.

2-Deoxy-*D*-Glucose



$C_6H_{12}O_5$ Mol Wt. 164.2

2-Deoxy-D-Glucose is 2-Deoxy Glucose.

2-Deoxy-D-Glucose contains not less than 98.0 per cent and not more than 102.0 per cent of $C_6H_{12}O_5$, calculated on the anhydrous basis.

Description. A white to off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *2-deoxy-D-glucose IPRS* or with the reference spectrum of 2-deoxy-D-glucose.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +44.0° to +48.0°, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in *water*, with the aid of ultrasound, and dilute to 5.0 ml with *water*.

Reference solution (a). A 2.0 per cent w/v solution of *glucose impurity (D- glucose) IPRS* in *water*.

Reference solution (b). Dissolve 100 mg of *2-deoxy-D-glucose IPRS* in 3.0 ml of *water*, with the help of cyclomixer, add 25 µl of reference solution (a) and dilute to 5.0 ml with *water*.

Reference solution (c). A 2.0 per cent w/v solution of *2-deoxy-D-glucose IPRS* in *water*. Dilute 1.0 ml of the solution to 100.0 ml with *water*. Dilute 1.0 ml of the solution to 10.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Inert sustain AQ-C18),

- column temperature: 55°,
- mobile phase: *water*,
- flow rate: 0.5 ml per minute,
- refractive index detector,
- detector cell temperature: 50°,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Glucose impurity[1]	0.86	—
2-Deoxy-*D*-glucose	1.0	—
Glucol impurity[2]	2.03	1.3
Furan diol impurity[3]	5.01	—

[1](3*R*,4*S*,5S,6*R*)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2,3,4,5-tetraol (or) Dextrose anhydrous (or) D-Glucose,

[2] (2*R*,3*S*,4*R*)-2-(hydroxymethyl)-3,4-dihydro-2*H*-pyran-3,4-diol,

[3]1-(furan-2-yl)ethane-1,2-diol.

Inject reference solution (b) and (c). The test is not valid unless the resolution between peaks due to glucose impurity and 2-deoxy-*D*-glucose is not less than 1.5 in the chromatogram obtained with reference solution (b) and the signal-to-noise ratio is not less than 15 in the chromatogram obtained with reference solution (c).

Inject reference solution (c) and the test solution. Run the chromatogram 10 times the retention time of the principal peak for test solution. The area of any peak corresponding to glucose impurity is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any peak corresponding to, each of, glucol impurity, furan diol impurity and any other secondary impurity is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 20 times the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.25 g of the substance under examination in *water,* with the aid of ultrasound, and dilute to 50.0 ml with *water.*

Reference solution. A 0.5 per cent w/v solution of *2-deoxy-D-glucose IPRS* in *water.*

Chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_6H_{12}O_5$.

Storage. Store protected from moisture.

2-Deoxy-*D*-Glucose Sachet

2-Deoxy-*D*-Glucose Sachet contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of 2-Deoxy-*D*-Glucose, $C_6H_{12}O_5$.

Usual strengths. 2.34 g; 5.85 g.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *2-deoxy-D-glucose IPRS* or with the reference spectrum of 2-deoxy-*D*-glucose.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +44.0° to +48.0°, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 5 sachets and disperse a quantity of mixed content containing 1.0 g of 2-deoxy-*D*-glucose in *water,* with the aid of vortex for 6 minutes, and dilute to 50.0 ml with *water.* Centrifuge at 10,000 RPM for 10 minutes and filter.

Reference solution. A 0.002 per cent w/v solution of *2-deoxy-D-glucose IPRS* in *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Inert sustain AQ-C18),
- column temperature: 55°,
- mobile phase: *water*,
- flow rate: 0.5 ml per minute,
- refractive index detector,
- detector cell temperature: 50°,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Glucose impurity[1*]	0.8	—
2-Deoxy-*D*-glucose	1.0	—
Glucol impurity[2*]	1.8	1.17
Furan diol impurity[3*]	4.3	—

*These are process impurities and are controlled in drug substances and no need to control in the formulation.

[1](3*R*,4*S*,5*S*,6*R*)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2,3,4,5-tetraol (or) Dextrose anhydrous (or) D-Glucose,

[2](2*R*,3*S*,4*R*)-2-(hydroxymethyl)-3,4-dihydro-2*H*-pyran-3,4-diol,

[3]1-(furan-2-yl)ethane-1,2-diol.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 20 times the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Other tests. Comply with the tests stated under Granules.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Seal test *(only for sachets)*. Loosely bundle 10 sachets with a rubber band and submerge the bundle under water in a vacuum desiccator maintained at a pressure not exceeding 18 kPa for one minute. Examine the bundle for any fine stream of bubbles. Re-establish normal pressure and open the bundle. No penetration of water is observed in any sachet.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 5 sachets and disperse a quantity of mixed content containing 0.25 g of 2-deoxy-*D*-glucose in *water*, with the aid of vortex for 6 minutes, and dilute to 250.0 ml with *water*. Centrifuge at 10,000 RPM for 10 minutes and filter.

Reference solution. A 0.1 per cent w/v solution of *2-deoxy-D-glucose IPRS* in *water*.

Chromatographic system as described under Related substances.

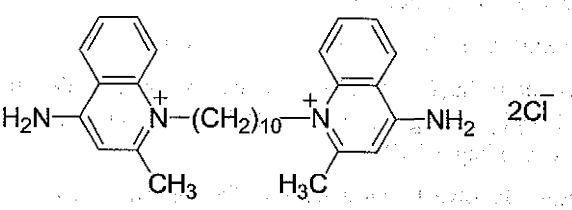
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_6H_{12}O_5$ in sachets.

Storage. Store protected from moisture.

Dequalinium Chloride



$C_{30}H_{40}Cl_2N_4$ Mol. Wt. 527.7

Dequalinium Chloride is 4,4′-diamino-2,2′-dimethyl-*N*,*N*′-decamethylenedi(quinolinium chloride).

Dequalinium Chloride contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{30}H_{40}Cl_2N_4$, calculated on the dried basis.

Category. Antiseptic.

Description. A creamy white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dequalinium chloride IPRS* or with the reference spectrum of dequalinium chloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0008 per cent w/v solution shows absorption maxima at 240 nm, 326 nm and 335 nm; absorbance at 240 nm, about 0.65, at 326 nm, about 0.4 and at 335 nm, about 0.35.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Acidity or alkalinity. Shake 0.1 g for 10 minutes with 100 ml of *carbon dioxide-free water* and add 0.5 ml of *bromocresol purple solution*. Not more than 0.2 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the solution.

Non-quaternised amines. Not more than 1.0 per cent, calculated as 4-aminoquinaldine, $C_{10}H_{10}N_2$, on the dried basis and determined by the following method. Shake 1.0 g with 45 ml of *water* for 5 minutes, add 5 ml of *dilute nitric acid* and shake for 10 minutes. Filter through cotton wool. Transfer 20.0 ml of the filtrate to a separator, add 20 ml of *1 M sodium hydroxide*, extract with two quantities, each of 50 ml, of *ether*, washing each extract in turn with the same 5 ml of *water*, and

then extract each ether extract successively with 20 ml, 20 ml and 5 ml of *1 M hydrochloric acid*. Combine the acid extracts, dilute to 50.0 ml with *1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 319 nm and 326.5 nm (2.4.7). The ratio of the absorbance at 319 nm to that at 326.5 nm is not less than 1.0. Calculate the percentage of $C_{10}H_{10}N_2$ from the expression 0.387a - 0.306b, where a and b are the specific absorbances at about 319 nm and 326.5 nm respectively.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

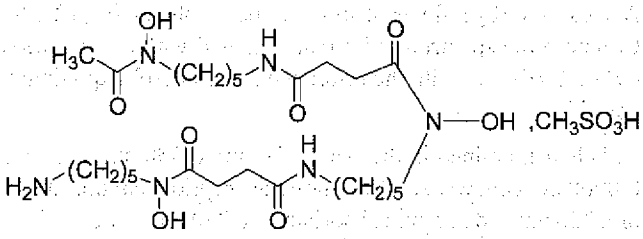
Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours at a pressure not exceeding 0.7 kPa.

Assay. Dissolve 0.5 g in a mixture of 80 ml of *anhydrous glacial acetic acid* and 20 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02638 g of $C_{30}H_{40}Cl_2N_4$.

Desferrioxamine Mesylate

Deferoxamine Mesylate; Deferoxamine Mesilate; Desferrioxamine Mesilate



$C_{25}H_{48}N_6O_8,CH_4SO_3$ Mol. Wt. 656.8

Desferrioxamine Mesylate is 30-amino-3,14,25-trihydroxy-3,9,14,20,25-pentaazatriacontane-2,10,13,21,24-pentaone methanesulphonate.

Desferrioxamine Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{25}H_{48}N_6O_8,CH_4SO_3$, calculated on the anhydrous basis.

Category. Iron-chelating agent.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *desferrioxamine mesylate IPRS* or with the reference spectrum of desferrioxamine mesylate. If the spectra obtained show differences, dissolve the substance under examination and the reference substance separately in *ethanol (95 per cent)*, evaporate to dryness and record new spectra using the residues.

B. Dissolve 5 mg in 5 ml of *water*, add 2 ml of a 0.5 per cent w/v solution of *tribasic sodium phosphate*, mix and then add 0.5 ml of a 2.5 per cent w/v solution of *sodium1,2-naphthoquinone-4-sulphonate*; a blackish brown colour is produced.

C. Dissolve 0.1 g in 5 ml of *2 M hydrochloric acid* and add 1 ml of *barium chloride solution*; the solution remains clear. In a porcelain crucible mix 0.1 g with 1 g of *anhydrous sodium carbonate*, heat and ignite over a Bunsen flame. Allow to cool, dissolve the residue in 10 ml of *water* by heating if necessary and filter; the filtrate gives reaction (A) of sulphates (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1), and the absorbance of the solution at about 425 nm is not more than 0.10 (2.4.7).

pH (2.4.24). 3.7 to 5.5, determined in a freshly prepared 10.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). Dissolve 10 mg of *deferoxamine mesilate IPRS* in 10.0 ml of the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution in 25.0 ml of the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: dissolve 1.32 g of *ammonium phosphate* and 0.37 g of *sodium edetate* in 950 ml of *water*, adjusted to pH 2.8 with *orthophosphoric acid* and 55 volumes of *tetrahydrofuran*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks with the relative retention time of about 0.8 and the principal peak is not less than 1.0.

Inject reference solution (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of secondary peak corresponding to deferoxamine mesilate impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (4.0 per cent), the sum of all the secondary peaks is not more than 1.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (7.0 per cent). Ignore any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.08 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Chlorides (2.3.12). 0.75 g complies with the limit test for chlorides (330 ppm).

Sulphates (2.3.17). 0.25 g complies with the limit test for sulphates (600 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

Assay. Weigh 50 mg of the substance under examination in 50.0 ml of *water*. To 2 ml of the solution, add 3 ml of *ferric chloride* solution prepared by dissolving 6.7 g of *ferric chloride* in 100 ml of 1 per cent v/v solution of *hydrochloric acid* and dilute to 25 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 485 nm (2.4.7). Calculate the content of $C_{25}H_{48}N_6O_8,CH_4O_3S$ from the absorbance obtained from a solution of known concentration of *deferoxamine mesylate IPRS*.

Desferrioxamine Mesylate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.025 Endotoxin Unit per mg of desferrioxamine.

Desferrioxamine Mesylate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light in a refrigerator (2° to 8°). Do not freeze. If the substance is sterile, store in sterile, air-tight, tamper-evident containers sealed so as to exclude micro-organisms.

Labelling. The label states where applicable, that the substance is sterile.

Desferrioxamine Injection

Desferrioxamine Mesylate Injection; Desferrioxamine Mesilate Injection

Desferrioxamine Injection is a sterile material consisting of Desferrioxamine Mesylate with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Desferrioxamine Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of desferrioxamine mesylate, $C_{25}H_{48}N_6O_8,CH_4SO_3$.

Usual strength. 0.5 g.

Description. A white or almost white powder; very hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

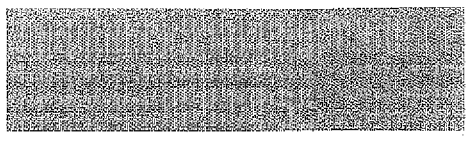
A. Dissolve 40 mg of the contents of the sealed container in 2 ml of *absolute ethanol* by heating on a water-bath at 60°, cool in ice until the substance begins to crystallise and evaporate to dryness at room temperature under a gentle current of nitrogen. Determine by infrared absorption spectrophotometry (2.4.6) on the residue. Compare the spectrum with that obtained with *desferrioxamine mesylate IPRS* or with the reference spectrum of desferrioxamine mesylate.

B. In the test for related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (b).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.



Test solution. Dissolve a quantity of injection containing 75 mg of Desferrioxamine Mesilate in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 25.0 ml with the mobile phase.

Reference solution (b). A 0.15 per cent w/v solution of *desferrioxamine mesilate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (10 µm) (Such as Nucleosil C18),
- mobile phase: a mixture of 95 volumes of solution containing 0.039 per cent w/v of *disodium edetate* and 0.139 per cent w/v of *ammonium phosphate*, adjusted to pH 2.8 with *orthophosphoric acid* and 5.5 volumes of *tetrahydrofuran*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks with the relative retention time of about 0.8 and the principal peak is not less than 1.0.

Inject the test solution and reference solution (a). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent) and the sum of all secondary peaks is not more than 1.75 times the area of the principal peak in the chromatogram obtained with reference solution (a) (7.0 per cent). Ignore any peak with an area less than 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.08 per cent).

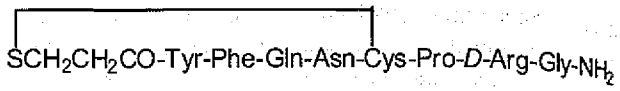
Bacterial endotoxins (2.2.3). Not more than 0.025 Endotoxin Unit per mg of desferrioxamine.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Dissolve a quantity of powder containing 50 mg of desferrioxamine mesylate in *water* and dilute to 50.0 ml with *water*. To 2 ml of the solution, add 3 ml of *ferric chloride solution* prepared by dissolving 6.7 g of *ferric chloride* in 100 ml of 1 per cent v/v solution of *hydrochloric acid* and dilute to 25.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at 485 nm (2.4.7). Calculate the content of $C_{25}H_{48}N_6O_8,CH_4O_3S$ in the injection from the absorbance obtained from a solution of known concentration of *desferrioxamine mesylate IPRS*.

Storage. Store protected from light at a temperature not exceeding 30°.

Desmopressin



$C_{46}H_{64}N_{14}O_{12}S_2$ Mol. Wt. 1069.2

Desmopressin is 1-Desamino,8-*D*-arginine vasopressin.

Desmopressin contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{46}H_{64}N_{14}O_{12}S_2$, calculated on the anhydrous and acetic acid free basis.

Category. Antidiuretic hormone.

Description. A white to off-white, fluffy powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

B. Amino acid analysis (2.2.19).

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, arginine and phenylalanine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; arginine: 0.90 to 1.10; phenylalanine: 0.90 to 1.10; tyrosine: 0.70 to 1.05; half-cystine: 0.30 to 1.05. Lysine, isoleucine and leucine are absent; not more than traces of other amino acids are present.

Tests

Specific optical rotation (2.4.22). –82.0° to –72.0°, determined in a 0.2 per cent w/v solution in 1.0 per cent v/v solution of *glacial acetic acid*, calculated on anhydrous and acetic acid free basis.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 1 mg of the substance under examination in 2.0 ml of *water*.

Reference solution. Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture IPRS* in 500 µl of *water*.

Chromatographic system

- a stainless steel column 12 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a *0.067 M phosphate buffer solution pH 7.0*,
  B. a mixture of equal volumes of *acetonitrile* and mobile phase A,
- a gradient programme using the conditions given below,

- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	76	24
4	76	24
18	58	42
35	48	52
40	76	24
45	76	24

The retention time of Desmopressin peak is about 16 minutes and of oxytocin peak is about 17 minutes.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to desmopressin and oxytoxin is not less than 1.5.

Inject the test solution, the area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.5 per cent. Ignore any peak with an area less than 0.05 per cent, calculated by area normalization.

Acetic acid. 3.0 to 8.0 per cent.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 5 volumes of mobile phase B and 95 volumes of mobile phase A.

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *glacial acetic acid* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: A. dilute 0.7 ml of *orthophosphoric acid* to 1000 ml with *water*, adjusted to pH 3.0 with *sodium hydroxide solution*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
5	95	5
10	50	50
20	50	50
22	95	5
30	95	5

The retention time of the peak corresponding to acetic acid is about 3 to 4 minutes.

Inject the reference solution and the test solution.

Calculate the content of acetic acid in the peptide.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.02 g.

Assay. Determine by liquid chromatography (2.4.14), as described under test for Related substances with the following modifications.

Reference solution. A 0.05 per cent w/v solution of *desmopressin IPRS* in *water.*

Chromatographic system

- mobile phase: a mixture of 40 volumes of mobile phase B and 60 volumes of mobile phase A,
- flow rate: 2 ml per minute,

The retention time of desmopressin is about 5 minutes.

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$.

Desmopressin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirements.

Bacterial endotoxins (2.2.3). Not more than 500 Endotoxin Units per mg of desmopressin.

Storage. Store protected from light and moisture at a temperature of 2° to 8°. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

Labelling. The label states: the mass of peptide per container; where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Desmopressin Intranasal Solution

Desmopressin Intranasal Solution is a solution of Desmopressin containing suitable buffering agents and preservatives.

Desmopressin Intranasal Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of the desmopressin, $C_{46}H_{64}N_{14}O_{12}S_2$.

Usual strength. 10 µg per ml.

Description. A colourless solution.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.5 to 5.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the intranasal solution in *water* to produce a final concentration of 0.0025 per cent w/v of the peptide.

Reference solution. Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture IPRS* in 10 ml of *water*.

Chromatographic system
- a stainless steel column 12 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *0.067 M mixed phosphate buffer pH 7.0*,
  B. a mixture of 10 volumes of *acetonitrile* and 10 volumes of mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 200 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	76	24
4	76	24
18	58	42
35	48	52
40	76	24
50	76	24

The retention time of desmopressin peak is about 16 minutes and of oxytocin is about 17 minutes.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is not less than 1.5 and the peak due to desmopressin is clearly separated from the peak due to the antimicrobial preservative stated on the label.

Inject the test solution. The area of any secondary peak is not more than 4.0 per cent and the sum of areas of all the secondary peaks is not more than 5.0 per cent. Ignore any peak with an area less than 0.3 per cent, calculated by area normalisation.

Other tests. Comply with the tests stated under Nasal Preparations.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the intranasal solution in *water* to give a final concentration of 0.0025 per cent w/v of the peptide.

Reference solution (a). A 0.0025 per cent w/v solution of *desmopressin IPRS* in *water*.

Reference solution (b). Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture IPRS* in 1.0 ml of *water*.

Chromatographic system
- a stainless steel column 12 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *acetonitrile* and 80 volumes of *0.067 M mixed phosphate buffer pH 7.0*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 200 µl.

The retention time of desmopressin peak is about 5 minutes.

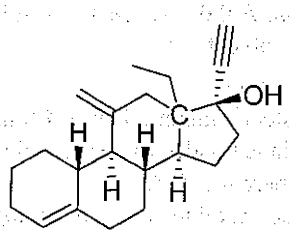
Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 1.5 and the peak due to desmopressin is clearly separated from the peak due to the antimicrobial preservative stated on the label.

Inject the reference solution and the test solution.

Calculate the content of $C_{46}H_{64}N_{14}O_{12}S_2$ in the intranasal solution.

Storage. Store protected from light, at a temperature of 2° to 8°.

Desogestrel



$C_{22}H_{30}O$ Mol. Wt. 310.5

Desogestrel is 18,19-Dinorpregn-4-en-20-yn-17-ol,13-ethyl-11-methylene-,(17α).

Desogestrel contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{30}O$, calculated on the dried basis.

Category. Steroidal hormone.

Description. A white or almost-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *desogestrel IPRS* or with the reference spectrum of desogestrel.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (d).

Tests

Specific optical rotation (2.4.22). + 53.0° to + 57.0°, determined in a 1.0 per cent w/v solution in *ethanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Dissolve 40 mg of the substance under examination in 50 ml of *acetonitrile* and dilute to 100.0 ml with *water*.

Reference solution (a). Dissolve 4 mg, each of, *desogestrel related compound A IPRS* and *desogestrel related compound D IPRS* in 50 ml of *acetonitrile*, and dilute to 100.0 ml with *water*.

Reference solution (b). Dissolve 40 mg of *desogestrel IPRS* in 50 ml of *acetonitrile*, add 1.0 ml of reference solution (a) and dilute to 100.0 ml with *water*.

Reference solution (c). Dissolve 4 mg, each of, *desogestrel IPRS, desogestrel related compound B IPRS* and *desogestrel related compound C IPRS* and 8 mg, each of, *desogestrel related compound A IPRS* and *desogestrel related compound D IPRS* in 50 ml of *acetonitrile*, dilute to 100.0 ml with *water*. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (d). Dissolve 40 mg of *desogestrel IPRS* in 50 ml of *acetonitrile* and dilute to 100.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 73 volumes of *acetonitrile* and 27 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 15 µl.

Name	Relative retention time
Desogestrel related compound B[1]	0.16
Desogestrel related compound C[2]	0.19
11-Methylene lynestrenol[3]	0.71
Desogestrel related compound A[4]	0.96
Desogestrel	1.0
Desogestrel related compound D[5]	1.06

[1]13-Ethyl-3-hydroxy-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol,

[2]13-Ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol-3-one,

[3]11-Methylene-19-nor-17α-pregn-4-en-20-yn-17-ol,

[4]13-Ethyl-11-methylene-18,19-dinor-5α,17α-pregn-3-en-20-yn-17-ol,

[5]13-Ethyl-11-methylenegon-4-en-17-one.

Inject reference solution (b) and (c). The test is not valid unless the resolution between the peaks due to desogestrel and desogestrel related compound A is not less than 1.3, the peak to valley ratio between the peaks due to desogestrel and desogestrel related compound D is not less than 2.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 5.0 per cent, for all peaks in the chromatogram obtained with reference solution (c).

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to desogestrel related compound B and desogestrel related compound C, each of, is not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (c) (0.1 per cent), the area of any peak corresponding to desogestrel related compound A and desogestrel related compound D, each of, is not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (c) (0.2 per cent), the area of any peak corresponding to 11-methylene lynestrenol is not more than twice the area of desogestrel peak in the chromatogram obtained with reference solution (c) (0.2 per cent), the area of any other secondary peak is not more than the area of the desogestrel peak in the chromatogram obtained with reference solution (c) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the desogestrel peak in the chromatogram obtained with reference solution (c) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the desogestrel peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent. Dry under vacuum at a pressure not exceeding 15 mm of mercury.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (b) and (d). The test is not valid unless the resolution between the peaks due to desogestrel and desogestrel related compound A is not less than 1.3, the peak-to-valley ratio between the peaks due to desogestrel and desogestrel related compound D is not less than 2.0 in the chromatogram obtained with reference solution (b), the tailing

factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent in the chromatogram obtained with reference solution (d).

Inject reference solution (d) and the test solution.

Calculate the content of the $C_{22}H_{30}O$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Desogestrel and Ethinyl Estradiol Tablets

Desogestrel and Ethinyl Estradiol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of desogestrel, $C_{22}H_{30}O$ and ethinyl estradiol, $C_{20}H_{24}O_2$.

Usual strengths. Desogestrel, 0.025 mg and Ethinyl Estradiol, 0.04 mg; Desogestrel, 0.1 mg and Ethinyl Estradiol, 0.03 mg; Desogestrel, 0.125 mg and Ethinyl Estradiol, 0.03 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of 0.05 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and centrifuge.

Determine by liquid chromatography (2.4.14).

Test solution. Use the clear supernatant liquid.

Reference solution (a). A 0.025 per cent w/v solution of *desogestrel IPRS* in *methanol*. Dilute 1.0 ml of the solution to 50.0 ml with the dissolution medium.

Reference solution (b). A 0.025 per cent w/v solution of *ethinyl estradiol IPRS* in *methanol*. Dilute 1.0 ml of the solution to 50.0 ml with the dissolution medium.

Reference solution (c). Dilute a suitable volume of reference solution (a) and reference solution (b) with dissolution medium to obtain a solution having similar concentration to the test solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with phenyl groups bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 2.72 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 6.0 with *2M sodium hydroxide* and 50 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer, set at 210 nm for desogestrel and fluorescence detector excitation at 285 nm and emission at 310 nm for ethinyl estradiol,
- injection volume: 200 µl.

The relative retention time with reference to desogestrel for ethinyl estradiol is about 0.2.

Inject reference solution (c). The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent for both the peaks.

Inject reference solution (c) and the test solution.

Calculate the contents of $C_{22}H_{30}O$ and $C_{20}H_{24}O_2$, in the medium.

Q. Not less than 80 per cent of the stated amounts of $C_{22}H_{30}O$ and $C_{20}H_{24}O_2$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one intact tablet in the solvent mixture, with the aid of mechanical shaker for 30 minutes and dilute to 25.0 ml with the solvent mixture. Dilute a suitable volume of the solution to obtain a solution containing 0.00005 per cent w/v of Desogestrel.

Inject reference solution (c) and the test solution.

Calculate the contents of $C_{22}H_{30}O$ and $C_{20}H_{24}O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

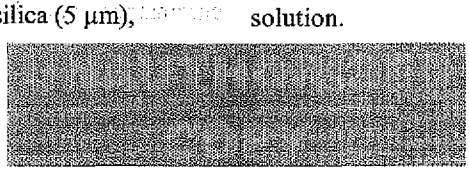
Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Disperse 10 intact tablets in 60 ml of the solvent mixture, with the aid of mechanical shaker for 30 minutes, and dilute to 100.0 with the solvent mixture, centrifuge and dilute clear supernatant liquid with the solvent mixture to obtain a solution containing 0.00005 per cent w/v of desogestrel.

Reference solution (a). A 0.025 per cent w/v solution of *desogestrel IPRS* in *methanol*.

Reference solution (b). A 0.03 per cent w/v solution of *ethinyl estradiol IPRS* in *methanol*.

Reference solution (c). Dilute a suitable volume of reference solution (a) and reference solution (b) with the solvent mixture to obtain a solution having similar concentration to the test solution.



Use chromatographic system as described under Dissolution.

Inject reference solution (c). The test is not valid unless the tailing factor is not more than 2.0 per cent and relative standard deviation for replicate injections is not more than 2.0 per cent for both the peaks.

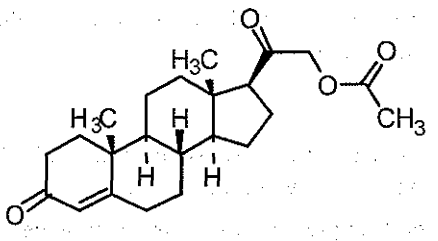
Inject reference solution (c) and the test solution.

Calculate the content of $C_{22}H_{30}O$ and $C_{20}H_{24}O_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Desoxycortone Acetate

Desoxycorticosterone Acetate; Deoxycorticosterone Acetate; Deoxycortone Acetate



$C_{23}H_{32}O_4$ Mol. Wt. 372.5

Desoxycortone Acetate is 3,20-dioxo-4-pregnen-21-yl acetate.

Desoxycortone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{23}H_{32}O_4$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white or creamy-white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, D and E may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *desoxycortone acetate IPRS* or with the reference spectrum of desoxycortone acetate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at 240 nm; absorbance at 240 nm, 0.43 to 0.46.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 90 volumes of *acetone* and 10 volumes of *1,2-propanediol*.

Mobile phase. A mixture of equal volumes of *cyclohexane* and *light petroleum (40° to 60°)*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *desoxycortone acetate IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the liquid to ascend to the top, remove the plate from the tank and allow the solvents to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

D. Dissolve 40 mg in 1 ml of *methanol*, warm and add 1 ml of *alkaline cupritartrate solution*; a red precipitate is formed.

E. Dissolve 5 mg in 0.5 ml of *methanol*, add 0.5 ml of *ammoniacal silver nitrate solution*; a black precipitate is slowly produced in the cold but is rapidly produced on warming.

Tests

Specific optical rotation (2.4.22). +171.0° to +179.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a). Dissolve 2 mg of *desoxycortone acetate IPRS* and 2 mg of *betamethasone 17-valerate IPRS* in the mobile phase and dilute to 200 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 200 ml with the mobile phase.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 350 ml of *water* and 600 ml of *acetonitrile*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Inject reference solution (a). The retention times are: betamethasone 17-valerate, about 7.5 minutes and desoxycortone acetate about 9.5 minutes. The test is not valid unless the resolution between the peaks corresponding to betamethasone 17-valerate and desoxycortone acetate is at least 4.5. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject reference solution (b) and the test solution. Continue the chromatography for three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the peaks other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.1 g in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution at the maximum at 240 nm (2.4.7). Calculate the content of $C_{23}H_{32}O_4$ taking 450 as the specific absorbance at 240 nm.

Storage. Store protected from light.

Desoxycortone Acetate Injection

Desoxycorticosterone Acetate Injection; Deoxycortone Acetate Injection

Desoxycortone Acetate Injection is a sterile solution of Desoxycortone Acetate in Ethyl Oleate or other suitable ester, in a suitable fixed oil, or in any mixture of these. It may contain suitable alcohols.

Desoxycortone Acetate Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of desoxycortone acetate, $C_{23}H_{32}O_4$.

Usual strength. 5 mg per ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 70 volumes of *n-heptane* and 30 volumes of *acetone*.

Test solution. Dilute the injection with *carbon tetrachloride* to give a solution containing 0.25 per cent w/v of Desoxycortone Acetate.

Reference solution. A 0.25 per cent w/v solution of *desoxycortone acetate IPRS* in *carbon tetrachloride*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable, spray with *ethanolic sulphuric acid (10 per cent v/v)*, heat at 105° for 30 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Ignore any spots due to the vehicle.

Tests

Bacterial endotoxins (2.2.3). Not more than 71.4 Endotoxin Units per mg of desoxycortone acetate.

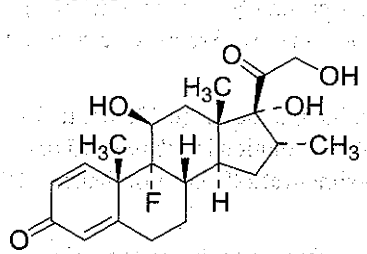
Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume containing 10 mg of Desoxycortone Acetate add sufficient *ethanol* to produce 100.0 ml. Dilute 10.0 ml of the solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 240 nm. Calculate the content of $C_{23}H_{32}O_4$ taking 450 as the specific absorbance at 240 nm.

Storage. Store protected from light.

Labelling. The label states (1) the composition of the solvent; (2) that it is meant for intramuscular injection only; (3) that any sediment should be dissolved by warming before use.

Dexamethasone



$C_{22}H_{29}FO_5$ Mol. Wt. 392.5

Dexamethasone is 9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione.

Dexamethasone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{22}H_{29}FO_5$, calculated on the dried basis.

Category. Adrenocortical steroid (anti-inflammatory).

Description. White or almost white crystals or a crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dexamethasone IPRS* or with the reference spectrum of dexamethasone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at about 254 nm.

Solvent mixture. 9 volumes of *chloroform* and 1 volume of *methanol*.

Mobile phase. A mixture of 85 volumes of *ether*, 10 volumes of *toluene* and 5 volumes of *1-butanol* saturated with *water*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). A 0.25 per cent w/v solution of *dexamethasone IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.125 per cent w/v each of the substance under examination and *dexamethasone IPRS* in the solvent mixture.

Reference solution (c). A solution containing 0.125 per cent w/v each of *dexamethasone IPRS* and *betamethasone IPRS* in the solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent v/v)*, heat at 120° for 10 minutes or until spots appear, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in day-light, fluorescence under ultraviolet light at 365 nm, in position and size to that in the chromatogram obtained with reference solution (a) and the chromatogram obtained with reference solution (b) shows only one spot. The test is not valid unless the chromatogram obtained with reference solution (c) shows two spots that are close to one another but separated.

C. Place 2 ml of a 0.01 per cent w/v solution in *ethanol* in a stoppered tube, add 10 ml of *phenylhydrazine-sulphuric acid solution*, mix, place in a water-bath at 60° for 20 minutes and cool immediately. Absorbance of the resulting solution at the maximum at about 419 nm, not less than 0.4 (2.4.7).

D. To 2 ml of *sulphuric acid* add 2 mg and shake to dissolve; a faint reddish brown colour is produced within 5 minutes. Add 10 ml of *water* and mix; the colour is discharged.

Tests

Specific optical rotation (2.4.22). +75.0° to +80.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 240 nm, 0.38 to 0.41.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. To 25 mg of the substance under examination add 1.5 ml of *acetonitrile* and 5 ml of mobile phase A. Mix with the aid of ultrasonic bath until the solids are completely dissolved and add sufficient of mobile phase A to produce 10 ml and mix well.

Reference solution (a). Dissolve 2 mg of *dexamethasone IPRS* and 2 mg of *methylprednisolone IPRS* in sufficient of mobile phase A to produce 100 ml.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: A. 25 volumes of *acetonitrile* and 70 volumes of *water* mixed, allowed to equilibrate and adjusted to 100 volumes with *water* and mixed,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
15	100	0
40	0	100
41	100	0

Equilibrate the column for at least 30 minutes with mobile phase B and then with mobile phase A for 5 minutes. For subsequent operations use the conditions described from 40 to 46 minutes.

Inject reference solution (a). The retention times are; methylprednisolone about 11.5 minutes, and dexamethasone about 13 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and

dexamethasone is at least 2.8; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject mobile phase A as the blank, the test solution and reference solution (b). Record the chromatogram of the test solution for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent). Ignore any peak due to the blank and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Dissolve 0.1 g in sufficient *ethanol* to produce 100.0 ml and mix. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol* and mix well. Determine the absorbance of the resulting solution (2.4.7) at the maximum at about 238 nm. Calculate the content of $C_{22}H_{29}FO_5$ taking 394 as the specific absorbance at 238 nm.

Storage. Store protected from light.

Dexamethasone Tablets

Dexamethasone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dexamethasone, $C_{22}H_{29}FO_5$.

Usual strength. 0.5 mg.

Identification

Shake a quantity of the powdered tablets containing 20 mg of Dexamethasone with 50 ml of *chloroform* for 30 minutes, filter and evaporate the filtrate to dryness at 105° for 2 hours. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dexamethasone IPRS* or with the reference spectrum of dexamethasone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. To 2 ml of *sulphuric acid* add 2 mg and shake to dissolve; a faint reddish brown colour is produced within 5 minutes. Add 10 ml of *water* and mix; the colour is discharged.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. To a weighed quantity of the tablets containing 2.5 mg of Dexamethasone add 10 ml of *acetonitrile*, mix with the aid of ultrasound and filter through a 0.45 µm filter. Dilute 4 ml of the filtrate to 10 ml with *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b). Dissolve 2 mg of *dexamethasone IPRS* and 2 mg of *methylprednisolone IPRS* in mobile phase A and dilute to 100.0 ml with the same solvent.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature. 45°,
- mobile phase: A. 15 per cent v/v of *acetonitrile*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
15	100	0
40	0	100
41	100	0

Inject reference solution (b). When the chromatograms are recorded, the retention times are; methylprednisolone about 13 minutes, and dexamethasone about 16 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and dexamethasone is at least 2.8; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the sum of the areas of all secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak due to mobile phase A and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Test solution. To one tablet, add sufficient *methanol (50 per cent)* to produce a solution containing 0.0025 per cent w/v of Dexamethasone, shake for 10 minutes and filter through glass-fibre filter.

Reference solution. A solution containing 0.0025 per cent w/v of *dexamethasone IPRS* in *methanol (50 per cent)*.

Use chromatographic system as described under Assay.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), protected from light.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 2.5 mg of Dexamethasone add 20.0 ml of *methanol (50 per cent)*, shake for 20 minutes and filter through a glass-fibre filter paper (Such as Whatman GF/C).

Reference solution. A solution containing 0.0125 per cent w/v of *dexamethasone IPRS* in *methanol (50 per cent)*.

Chromatographic system

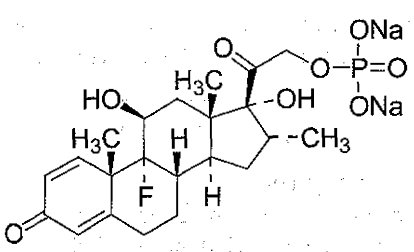
- a stainless steel column 20 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as (Spherisorb ODS 1),
- mobile phase: a mixture of 53 volumes of *water* and 47 volumes of *methanol*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{29}FO_5$ in the tablets.

Storage. Store protected from light.

Dexamethasone Sodium Phosphate



$C_{22}H_{28}FNa_2O_8P$ Mol. Wt. 516.4

Dexamethasone Sodium Phosphate is disodium 9α-fluoro-11β,17α-dihydroxy-16α-methyl-3,20-dioxo-1,4-pregnadien-21-yl phosphate.

Dexamethasone Sodium Phosphate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{22}H_{28}FNa_2O_8P$, calculated on the anhydrous, and ethanol-free or solvent-free basis.

Category. Adrenocortical steroid (anti-inflammatory).

Description. A white or slightly yellow, crystalline powder; very hygroscopic. It shows polymorphism (2.5.11).

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if Tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dexamethasone sodium phosphate IPRS* or with the reference spectrum of dexamethasone sodium phosphate.

B. Dissolve 10 mg in 5 ml of *water* and dilute to 100 ml with *ethanol*. To 2 ml of the resulting solution in a glass-stoppered tube add 10 ml of *phenylhydrazine- sulphuric acid solution*, mix, heat in a water-bath at 60° for 20 minutes and cool immediately. Absorbance of the resulting solution at the maximum at about 419 nm, not less than 0.20 (2.4.7).

C. In the test for Related substances, the principal peak in the chromatogram obtained with reference solution (b) corresponds to the peak in the chromatogram obtained with reference solution (c).

D. Heat gently 40 mg with 2 ml of *sulphuric acid* until white fumes are evolved, add *nitric acid* dropwise until oxidation is complete and cool. Add 2 ml of *water*, heat until white fumes are evolved again, cool, add 10 ml of *water* and neutralise to *litmus paper* with *5 M ammonia*. The solution gives reaction (A) of sodium salts and reaction (B) of phosphates (2.3.1).

Tests

pH (2.4.24). 7.5 to 9.5, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). +75.0° to +83.0°, determined in a 1.0 per cent w/v solution.

Inorganic phosphates. Not more than 0.5 per cent, calculated as PO_4, determined by the following method. Weigh 25 mg, dissolve in 10 ml of *water*, add 4 ml of *dilute sulphuric acid*, 1 ml of *ammonium molybdate solution* and 2 ml of *methylaminophenol* with *sulphite solution* and allow to stand for 15 minutes. Add sufficient *water* to produce 25.0 ml, allow to stand for further 15 minutes and measure the absorbance of the resulting solution at the maximum at 730 nm (2.4.7). Calculate the content of phosphate from a calibration curve prepared by treating suitable aliquots of a 0.00143 per cent w/v solution of *potassium dihydrogen phosphate* in a similar manner.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (a). Dissolve 2 mg of *dexamethasone sodium phosphate IPRS* and 2 mg of *betamethasone sodium phosphate IPRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (c). A 0.0025 per cent w/v solution of *dexamethasone sodium phosphate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1.360 g of *potassium dihydrogen phosphate* and 0.60 g of *hexylamine* allowed to stand for 10 minutes and then dissolved in 182.5 ml of *water* and 67.5 ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 45 minutes.

The retention times of betamethasone sodium phosphate is about 12.5 minutes and dexamethasone sodium phosphate is about 14 minutes.

Inject reference solution (a). The test is not valid unless the resolution between the peaks corresponding to betamethasone sodium phosphate and dexamethasone sodium phosphate is at least 2.2. If necessary, adjust the concentration of acetonitrile or increase the concentration of water in the mobile phase.

Inject reference solution (b) and the test solution. Run the chromatogram for twice the retention time of the principal peak for the test solution, the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

Ethanol. Not more than 8.0 per cent w/w, determined by gas chromatography (2.4.13).

Internal Standard. A 1.0 per cent v/v solution of *1-propanol* in *water*.

Test solution. A 10.0 per cent w/v solution of the substance under examination in the internal standard solution.

Reference solution. A solution containing 1.0 per cent w/v of *ethanol* in the internal standard solution.

Chromatographic system.

- a capillary column 30 m x 0.25 mm packed with 6.0 per cent polycyanopropylphenyl siloxane and 94.0 per cent of polydimethyl siloxane (1.4 µm) (Such as DB-624),
- temperature:
  column 50° for 2 minutes, 50° to 240° @ 20° per minute and hold at 240° for 2 minutes,
- inlet port at 250° and detector at 280°,
- flow rate: 0.5 ml per minute, using nitrogen as carrier gas,
- flame ionization detector,
- split ratio: 25:1.

Inject 1 µl of each solution.

The resolution between the peaks due to ethanol and 1-propanol is not less than 2.0. The test is not valid unless the relative standard deviation of peak area ratio of ethanol and internal standard from replicate injections of reference solution is not more than 2.0 per cent.

Calculate the percentage w/w of ethanol assuming the weight per ml at 25° to be 0.787 g.

Total ethanol and water. Determine the content of water (2.3.43), using 0.2 g. Not more than 16.0 per cent w/w, calculated from the sum of the percentage of ethanol determined by the method described above and the percentage of water.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). Dissolve 2 mg each of *dexamethasone IPRS* (dexamethasone sodium phosphate impurity A) and *dexamethasone sodium phosphate IPRS* in 2 ml of *tetrahydrofuran*, then dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). A 0.006 per cent w/v solution of *dexamethasone sodium phosphate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (7 µm),
- mobile phase: mix 520 ml of *water* with 2 ml of *orthophosphoric acid*, adjusted to pH 2.6 with *sodium hydroxide*. Mix this solution with 36 ml of *tetrahydrofuran* and 364 ml of *methanol*,

– flow rate: 1.5 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 20 µl.

The relative retention time with reference to dexamethasone sodium phosphate (retention time: about 8 minutes) for dexamethasone sodium phosphate impurity A is about 2.0.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to dexamethasone sodium phosphate and dexamethasone sodium phosphate impurity A is not less than 6.0.

Inject reference solution (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak.

Calculate the content of $C_{22}H_{28}FNa_2O_8P$.

Storage. Store protected from light.

Dexamethasone Injection

Dexamethasone Sodium Phosphate Injection

Dexamethasone Injection is a sterile solution of Dexamethasone Sodium Phosphate in Water for Injections.

Dexamethasone Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dexamethasone phosphate, $C_{22}H_{30}FO_8P$.

Usual strength. The equivalent of 4 mg of dexamethasone phosphate per ml. (4.4 mg of dexamethasone sodium phosphate is approximately equivalent to 4 mg of dexamethasone phosphate).

Description. A clear solution.

Identification

In the assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to dexamethasone sodium phosphate in the chromatogram obtained with the reference solution (a).

Tests

pH (2.4.24). 7.0 to 8.5.

Free dexamethasone. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection with the mobile phase to produce a solution containing the equivalent of 0.25 per cent w/v of dexamethasone phosphate.

Reference solution (a). A 0.00125 per cent w/v solution of *dexamethasone IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.25 per cent w/v of *dexamethasone sodium phosphate IPRS*, 0.01 per cent w/v of *propyl hydroxybenzoate* and 0.001 per cent w/v of *dexamethasone IPRS* in the mobile phase.

Reference solution (c). A solution containing 0.01 per cent w/v of *propyl hydroxybenzoate* in the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
– mobile phase: a mixture of 1.360 g of *potassium dihydrogen phosphate* and 0.60 g of *hexylamine* allowed to stand for 10 minutes and then dissolved in 182.5 ml of *water* and 67.5 ml of *acetonitrile*, mixed and filtered,
– flow rate: 1 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 45 minutes.

Inject reference solution (b). The test is not valid unless the peak corresponding to dexamethasone is completely separated from the peaks due to dexamethasone sodium phosphate and propyl hydroxybenzoate. If necessary, adjust the concentration of *acetonitrile* or increase the concentration of *water* in the mobile phase.

Inject the test solution and reference solution (a). Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak corresponding to dexamethasone is not greater than that of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Bacterial endotoxins (2.2.3). Not more than 31.3 Endotoxin Units per mg of dexamethasone phosphate.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a measured volume of the injection containing about 8 mg of dexamethasone phosphate to 100.0 ml with the mobile phase and mix.

Reference solution (a). A 0.008 per cent w/v solution of *dexamethasone sodium phosphate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v each of *dexamethasone sodium phosphate IPRS* and *betamethasone sodium phosphate IPRS* in the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),

- mobile phase: a mixture of 1.360 g of *potassium dihydrogen phosphate* and 0.6 g of *hexylamine* allowed to stand for 10 minutes and then dissolved in 182.5 ml of *water* and 67.5 ml of *acetonitrile*, mixed and filtered,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to betamethasone sodium phosphate and dexamethasone sodium phosphate is at least 2.2. If necessary, adjust the concentration of acetonitrile or increase the concentration of *water* in the mobile phase.

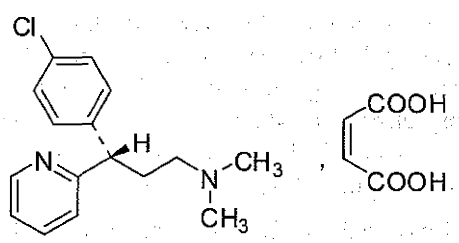
Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{30}FO_8P$ in the injection.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of dexamethasone phosphate in a suitable dose-volume.

Dexchlorpheniramine Maleate



$C_{16}H_{19}ClN_2,C_4H_4O_4$ Mol. Wt. 390.9

Dexchlorpheniramine Maleate is (3*S*)-3-(4-chlorophenyl)-*N*,*N*-dimethyl-3-(pyridin-2-yl)propan-1-amine maleate.

Dexchlorpheniramine Maleate contains not less than 98.0 per cent and not more than 100.5 per cent of $C_{16}H_{19}ClN_2,C_4H_4O_4$, calculated on the dried basis.

Category. Antihistaminic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dexchlorpheniramine maleate IPRS* or with the reference spectrum of dexchlorpheniramine maleate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *water* (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 4.5 to 5.5, determined in a 1 per cent w/v solution in *water*.

Specific optical rotation (2.4.22). +22° to +23°, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 95 volumes of mobile phase A and 5 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *dexchlorpheniramine maleate IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A 0.0002 per cent w/v solution of *chlorpheniramine related compound C IPRS* in reference solution (a).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 5.4 g of *monobasic potassium phosphate* in 900 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*;
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate:1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
1	95	5
20	70	30
30	70	30
31	95	5
40	95	5

Name		Relative retention time
Maleic acid	0.18	---
Chlorpheniramine related compound B[1]	0.49	---
Pheniramine	0.57	2.5
Chlorpheniramine related compound C[2]	0.97	---
Dexchlorpheniramine	1.0	---

[1]Di (pyridin-2-yl) amine. (for information purpose),
[2]3-(4-Chlorophenyl-*N*-methyl-3-(pyridin-2-yl) propan-1-amine. (used to establish system suitability only).

Inject reference solution (b) and (c). The test is not valid unless the resolution between the peaks due to chlorpheniramine related compound C and dexchlorpheniramine is not less than 1.5 in the chromatogram obtained with reference solution (c) and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak corresponding to pheniramine is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore the peak due to maleic acid and any peak with on area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 3 ml of *water*. Add a few drops of *ammonia* until an alkaline reaction is produced. Shake with 5 ml of *dichloromethane*. Separate the layers. Evaporate the lower, dichloromethane layer to an oily residue on a water-bath. Dissolve the oily residue in *2-propanol* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 10 mg of *dexchlorpheniramine maleate IPRS* in 3 ml of *water*. Add a few drops of *ammonia* until an alkaline reaction is produced. Shake with 5 ml of *dichloromethane*. Separate the layers. Evaporate the lower, dichloromethane layer to an oily residue on a water-bath. Dissolve the oily residue in *2-propanol* and dilute to 10.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of *chlorpheniramine maleate IPRS* in 3 ml of *water*. Add a few drops of *ammonia* until an alkaline reaction is produced. Shake with 5 ml of *dichloromethane*. Separate the layers. Evaporate the lower, dichloromethane layer to an oily residue on a water-bath. Dissolve the oily residue in *2-propanol* and dilute to 10.0 ml with the same solvent.

Reference solution (c). Dilute 1.0 ml of the test solution to 50.0 ml with *2-propanol*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with amylose derivative of silica,
- column temperature: 40°,
- mobile phase: a mixture of 0.3 volumes of *diethylamine*, 2.0 volumes of *2-propanol* and 98 volumes of *hexane*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to (R)-enantiomer and (*S*)-enantiomer is not less than 1.5.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to the (R)-enantiomer is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent); the area of any other secondary peak is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum oven at 65° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$.

Storage. Store protected from light.

Dexchlorpheniramine Oral Solution

Dexchlorpheniramine Maleate Oral Solution

Dexchlorpheniramine Oral Solution is a mixture consisting of Dexchlorpheniramine Maleate with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The oral solution is constituted by dispersing the contents of the sealed container in the specific volume of water just before use.

Dexchlorpheniramine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dexchlorpheniramine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$.

Usual strength. 2 mg per 5 ml.

Storage. Store the constituted solution in a refrigerator (2° to 8°). Discard any unused portion after 30 days of reconstitution.

The contents of the sealed container comply with the test requirements stated under Oral Liquids and with the following requirements.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the test solution and the reference solution prepared in the Assay, shows an absorption maximum only at 264 nm.

B. Evaporate the remaining extract from the Assay on a steam bath to a small volume, then transfer it to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3 ml portions of *dimethylformamide*, to a suitable glass-stoppered graduated cylinder, dilute with *dimethylformamide* to 15.0 ml, and mix, the optical rotation (2.4.22) of the solution is between +0.06° and +0.11°.

Tests

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Weigh a measured volume of oral solution, containing about 40 mg of Dexchlorpheniramine Maleate, in 250 ml of *water*, adjusted to pH 11.0 with *1 M sodium hydroxide*, cool. Extract with five 70 ml portions of *hexane*, combine the *hexane* extracts in a 500-ml separator, and wash the *hexane* with two 10 ml portions of *sodium hydroxide* (1 in 250). Extract the combined alkaline washings with two 20 ml portions of *hexane*, and add these extracts to the bulk of the alkali-washed *hexane*. Filter the hexane through a pledged of cotton that previously has been saturated with *hexane* into a 500-ml volumetric flask, rinse the separator with portions of solvent hexane, pass the rinsing through the filter to add to volume, and mix. Transfer 50.0 ml of the solution to a separator. Extract the *hexane* with two 40 ml portions of *dilute hydrochloric acid* (1 in 120), combine the acid extracts in a 100-ml volumetric flask, add *dilute hydrochloric acid* (1 in 120) to volume, and mix. Filter the solution into a glass-stoppered conical flask, discarding the first few ml of the filtrate. The concentration of dexchlorpheniramine maleate is about 40 µg per ml. Measure the absorbance of the resulting solution at the maximum at about 264 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ from the absorbance obtained by repeating the procedure, using 40 mg of *dexchlorpheniramine maleate IPRS* in place of the substance under examination.

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$, weight in volume.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Dexchlorpheniramine Tablets

Dexchlorpheniramine Maleate Tablets

Dexchlorpheniramine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dexchlorpheniramine maleate, $C_{16}H_{19}ClN_2,C_4H_4O_4$.

Usual strength. 2 mg.

Identification

A. Shake a quantity of powdered tablets containing about 50 mg of Dexchlorpheniramine Maleate with 25 ml of *0.01 M hydrochloric acid* for 10 minutes. Transfer the liquid to a separator, if necessary filter it and wash the filter and the residue with several small portions of *water*. In a second separator dissolve 50 mg of *dexchlorpheniramine maleate IPRS* in 25 ml of *0.01 M hydrochloric acid*. Treat each solution as follows. Add 2 ml of *1 M sodium hydroxide* and 4 ml of *carbon disulphide*, and shake for 2 minutes. Centrifuge if necessary to clarify the lower phase, and filter it through a dry filter, collecting the filtrate in a small flask provided with a glass stopper. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dexchlorpheniramine maleate IPRS* treated in the same manner or with the reference spectrum of dexchlorpheniramine.

B. Shake a quantity of finely powdered tablets containing about 150 mg of Dexchlorpheniramine Maleate with 100 ml of *1 M acetic acid* for 10 minutes, filter through a sintered-glass funnel into a suitable vessel, adjust the filtrate with 10 per cent w/v *sodium hydroxide solution* to a pH of 11, and extract the solution with six 100-ml portions of hexane, filtering each hexane extract using suitable means to effect separation of the hexane layer from the aqueous layer. Concentrate the combined extracts on a steam bath to a small volume, transfer to a smaller, more suitable vessel, and evaporate just to the point where hexane vapours are no longer perceptible. Transfer the oily residue, with the aid of four 3 ml portions of *dimethylformamide*, to a suitable glass-stoppered graduated cylinder, dilute with *dimethylformamide* to 15.0 ml, mix, and centrifuge if necessary: the optical rotation of the solution is between +0.24° and +0.35°.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 500 ml of *water,*

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by gas chromatography (2.4.13).

Internal standard solution. Dissolve 9 mg of the *dexbrompheniramine maleate IPRS* in 100 ml of *water.*

Test solution. Dilute 15.0 ml of the dissolution medium to 50 ml with *water,* add 1.0 ml of internal standard solution and mix. Adjust to pH 11.0 with 50 per cent w/v solution of *sodium hydroxide,* add 3.0 ml of *hexane* and sonicate for 3 minutes, centrifuge, and use the clear supernatant hexane layer.

Reference solution. A 0.00125 per cent w/v solution of *dexchlorpheniramine maleate IPRS* in *water.* Dilute 5.0 ml of the solution to 10.0 ml with *water,* add 1.0 ml of internal standard solution and mix. Adjust to pH 11.0 with 50 per cent w/v solution of *sodium hydroxide,* add 3.0 ml of *hexane* and sonicate for 3 minutes, centrifuge, and use the clear supernatant hexane layer.

Chromatographic system

- a glass column 1.8 m x 2 mm, packed with 1.2 per cent phase G16 and 0.5 per cent potassium hydroxide support with S1AB,
- temperature:
  column. 205°,
  inlet port and detector at 250°,
- flow rate: 60 ml per minute using nitrogen as carrier gas.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to dexchlorpheniramine and dexbrompheniramine is not less than 1.9 and the relative standard deviation for replicate injections is not more than 2 per cent. The relative retention time with reference to dexbrompheniramine for dexchlorpheniramine is about 0.7.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ in the tablet.

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{19}ClN_2,C_4H_4O_4$.

Uniformity of content. Complies with the test stated under Tablets.

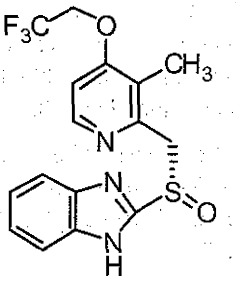
To 1 tablet, add 10 ml of *water* and shake, adjust to pH 11.0 with 10 per cent w/v solution of *sodium hydroxide,* Extract the mixture with two 7.5 ml portions of *hexane,* and combine the extracts in a separator. Extract the *hexane* with three 5.0 ml portions of *dilute hydrochloric acid* (1 in 120), combining the acid extracts in a 25-ml volumetric flask. Add dilute *hydrochloric acid* (1 in 120) to volume, and mix. Measure the absorbance of the resulting solution at the maximum at about 264 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ from the absorbance obtained by repeating the procedure, using 10 ml of 0.02 per cent w/v solution of *dexchlorpheniramine maleate IPRS* in place of the substance under examination.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 8.0 mg of Dexchlorpheniramine Maleate in 50 ml of *water,* adjusted to pH 11.0 with *sodium hydroxide* (1 in 10). Extract the mixture with two 75 ml portions of *hexane,* and combine the extracts in a separator. Extract the *hexane* with three 50 ml portions of *dilute hydrochloric acid* (1 in 120), combining the acid extracts in a 200-ml volumetric flask. Add dilute *hydrochloric acid* (1 in 120) to volume, and mix. Measure the absorbance of the resulting solution at the maximum at 264 nm (2.4.7). Calculate the content of $C_{16}H_{19}ClN_2,C_4H_4O_4$ from the absorbance obtained by repeating the procedure, using 10 ml of 0.08 per cent w/v solution of *dexchlorpheniramine maleate IPRS* in place of the substance under examination.

Storage. Store protected from moisture.

Dexlansoprazole



$C_{16}H_{14}F_3N_3O_2S$ Mol wt. 369.4 (anhydrous)

$C_{16}H_{14}F_3N_3O_2S,1½\,H_2O$ Mol wt. 396.4 (sesquihydrate)

Dexlansoprazole is (+)-2-[[4-(2,2,2-Trifluoroethoxy)-3-methylpyridin-2-yl]methylsulfinyl]-1*H*-benzo[d]imidazole or its sesquihydrate.

Dexlansoprazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{14}F_3N_3O_2S$, calculated on the anhydrous basis.

Category. Antiulcerative.

Description. A white to brownish white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dexlansoprazole IPRS* or with the reference spectrum of dexlansoprazole.

B. In the test for Related substances, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

S-isomer. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution(a). Weigh 25 mg of *S-isomer IPRS* and dissolve in mobile phase and diluted to50.0 ml with mobile phase.

Reference solution(b). Dissolve 25 mg of *dexlansoprazole IPRS* in mobile phase , add 75 µl of reference solution (a) and dilute to 50.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, such as chiral Pack- IC, (5 µm)
- column temperature 30°,
- mobile phase: a mixture of 1 volume of *trifluoro acetic acid* , 0.5 volume of *diethylamine* and 1000 volumes of *acetonitrile.*
- flow rate: 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

The test is not valid unless. The resolution between the dexlansoprazole and S -isomer (-)-2-[(*S*)-{[3-methyl-4-(2,2,2-trifluoroethoxy) pyridine-2-yl]methyl}sulfinyl]-1*H*-benzimidazole is not less than 2.0.

Inject the test solution, the area of any peak due to S-isomer is not mre than 0.15 per cent, calculated by area normalisation.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in *0.01 M methanolic sodium hydroxide* and diluted to 10.0 ml with *0.01 M methanolic sodium hydroxide* in amber coloured volumetric flask.

Reference solution. A 0.0001 per cent w/v solution of *dexlansoprazole IPRS* in *0.01 M methanolic sodium hydroxide* in amber coloured volumetric flask.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 30°,
- mobile phase: A. *water*,

B. a mixture of 160 volumes of *acetonitrile*, 40 volumes of *water* and 1 volume of *triethylamine*, adjusted to pH 7.0 with *orthophosphoric acid*,

- flow rate: 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
10	60	40
25	45	55
35	35	65
45	35	65
47	60	40
55	60	40

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0.

Inject the test solution, the area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent (for the anhydrous form) and 6.0 to 8.0 per cent (for the sesquihydrate form), determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

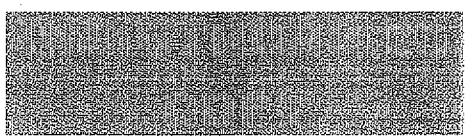
Solvent mixture. 1 volume of *triethylamine* with 60 volumes of *water* and adjusted to pH 10.5 using dilute *orthophosphoric acid.* Filter this solution through 0.45 µm nylon filter paper.

Test solution. Dissolve 0.1 g of the substance under examination in 50 ml of solvent mixture in 100-ml amber coloured volumetric flask and dilute to 100.0 ml with solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml in amber coloured volumetric flask with mobile phase.

Reference solution. A 0.01 per cent w/v solution of *dexlansoprazole IPRS* using amber coloured volumetric flask in the mobile phase.

Chromatographic system

- a stainless steel column 150 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: a mixture of 65 volumes of *water*, 35 volumes of *acetonitrile*, and 0.5 volume of *triethylamine* and adjusted to pH 7.0 with *orthophosphoric acid.*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 10 µl.



Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{14}F_3N_3O_2S$.

Dextran 1

Dextran 1 is a low molecular weight fraction of dextran, consisting of a mixture of isomaltooligosaccharide.It is obtained by controlled hydrolysis and fractionation of dextrans produced by fermentation of *Leuconostoc mesenteroides* in the presence of sucrose. It is a glucose polymer in which the linkages between glucose units are almost exclusively α-1,6. Its weight-average molecular weight is about 1000.

Category. Plasma substitute.

Description. A white to off white hygroscopic powder.

Identification

A. To 2 mg of substance under examination, add 2 drops of *water*, grind in an agate mortar for 2 minutes, add 0.3 g of *potassium bromide* and mix to slurry. (*NOTE — Do not grind).* Dry under vaccum at 40°. On the residue, determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *dextran 1 IPRS* treated in the same manner or with the reference spectrum of dextran 1.

B. Specific optical rotation (see Test).

C. Molecular mass distribution (See Test), the peaks in the chromatogram obtained with the test solution corresponds to peaks in the chromatogram obtained with reference solution.

Tests

pH(2.4.24). 4.5 to 7.0,determined in a15 per cent w/v solution.

Specific optical rotation (2.4.22). +148.0° to +164.0°, determined on 1.0 per cent w/v solution at 20°.

Light absorbance. A 15 per cent w/v solution, determined at 375 nm (2.4.7), shows absorbance not more than 0.12.

Molecular-mass distribution. Determined by size-exclusion chromatography (2.4.16).

Test solution. Dissolve 6 mg of the substance under examination in 1.0 ml of the mobile phase.

Reference solution (a). Dissolve 6 mg of *dextran 1 IPRS* in 1.0 ml of the mobile phase.

Reference solution (b). A solution containing 0.045 per cent w/v of isomaltotriose (3 glucose units), isomaltononaose (9 glucose units) and 0.060 per cent w/v of sodium chloride in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 10 mm, dextran covalently bound to highly cross-linked porous agarose beads, allowing resolution of oligosaccharides in the molecular mass range of 180 to 3000 (two columns coupled in series),
- temperature: 20-25°,
- mobile phase: a 0.292 per cent w/v solution of *sodium chloride*,
- flow rate: 0.07-0.08 ml per minute,
- differential refractometer,
- injection volume: 100 µl.

Identification of peaks, use the chromatogram obtained with reference solution (b) to identify the peaks due to isomaltotriose, isomaltononaose and sodium chloride.

Determine the peak areas. Disregard any peak due to sodium chloride. Calculate the average relative molecular mass M_w and the amount of the fraction with less than 3 and more than 9 glucose units, of *dextran 1 IPRS* and of the substance under examination, using the following expression:

$$M_w = \sum w_i \times m_i$$

where, M_w = average molecular mass of the dextran 1;
m_i = molecular mass of oligosaccharide *i*;
w_i = weight proportion of oligosaccharide *i*.

Use the following m_i values for the calculation:

Oligosaccharide *i*	m_i
glucose	180
isomaltose	342
isomaltotriose	504
isomaltotetraose	666
isomaltopentaose	828
isomaltohexaose	990
isomaltoheptaose	1152
isomaltooctaose	1314
isomaltononaose	1476
isomaltodecaose	1638
isomaltoundecaose	1800
isomaltododecaose	1962
isomaltotridecaose	2124
isomaltotetradecaose	2286
isomaltopentadecaose	2448
isomaltohexadecaose	2610
isomaltoheptadecaose	2772
isomaltooctadecaose	2934
isomaltononadecaose	3096

Inject reference solution (a). The test is not valid unless the values obtained for *dextran 1 IPRS* are within the values stated on the label.

The average molecular mass range between 850 and 1150; fraction with less than 3 glucose units less than 15.0 per cent and fraction with more than 9 glucose units less than 20.0 per cent.

Nitrogen-containing substances. Not more than 110 ppm of N.

Determine the content of nitrogen, method A (2.3.30), using 0.2 g and heating for 2 hours. Collect the distillate in a mixture of 0.5 ml of *bromocresol green solution*, 0.5 ml of *methyl red solution* and 20 ml of *water*. Titrate with *0.01 M hydrochloric acid*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Sodium chloride. Not more than 1.5 per cent.

Dissolve 5 g of the substance under examination in 100 ml of *water*. Titrate with *0.1 M silver nitrate*, using 0.2 ml of *potassium chromate* solution as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 5.844 mg of NaCl.

Residual solvents (5.4). Not more than 0.5 per cent *ethanol*, 0.05 per cent *methanol* and sum of solvents other than *ethanol*, *methanol* and *propanol* is not more than 0.5 per cent calculated as *propanol*.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 5 hours.

Dextran 1 intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Dextran 1 intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 25 Endotoxin Unit per g of dextran.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 10^2 CFU per g and the total combined moulds and yeasts count is not more than 10 CFU per g.

Storage. Store protected from moisture, at a temperature between 4° and 30°.

Labelling. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

Dextran 40

Dextran 40 is a mixture of polysaccharides, principally of the α-1, 6-glucan type. Average relative molecular mass is about 40,000. It is obtained by hydrolysis and fractionation of dextran produced by fermentation of sucrose using strain of *Leuconostoc mesenteroides*.

Category. Plasma substitute.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextran 40 IPRS* or with the reference spectrum of dextran.

B. Specific optical rotation (see Tests).

C. Molecular mass distribution (see Tests) the peaks in the chromatogram obtained with the test solution corresponds to peaks in the chromatogram obtained with reference solution.

Tests

Solution A. Dissolve 5 g in *water*, heating on a water bath and dilute to 50.0 ml with *water*.

Appearance of solution. Solution A is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A, add 0.1 ml of *phenolphthalein solution*, the solution remains colourless. Add 0.2 ml of *0.01 M sodium hydroxide*, the solution is pink. Add 0.4 ml of *0.01 M hydrochloric acid*, the solution is colourless, add 0.1 ml of *methyl red solution*, the solution is red or orange.

Specific optical rotation (2.4.22). + 195.0° to + 203.0°, determined on 2.0 per cent w/v solution.

Molecular-mass distribution. Determine by size-exclusion chromatography (2.4.16).

Test solution. Dissolve 6 mg of the substance under examination in 1.0 ml of the mobile phase.

Reference solution (a). Dissolve 6 mg of *dextran 40 IPRS* in 1.0 ml of the mobile phase.

Reference solution (b). A solution containing 0.045 per cent w/v of isomaltotriose (3 glucose units), isomaltononaose (9 glucose units) and 0.060 per cent w/v of sodium chloride in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 10 mm, dextran covalently bound to highly cross-linked porous agarose beads, allowing resolution of oligosaccharides in the molecular mass range of 180 to 3000 (two columns coupled in series),

- temperature: 20-25°,
- mobile phase: a 0.292 per cent w/v solution of *sodium chloride*,
- flow rate: 0.07-0.08 ml per minute,
- differential refractometer,
- injection volume: 100 µl.

Identification of peaks, use the chromatogram obtained with reference solution (b) to identify the peaks due to isomaltotriose, isomaltononaose and sodium chloride.

Determine the peak areas. Disregard any peak due to sodium chloride. Calculate the average relative molecular mass M_w and the amount of the fraction with less than 3 and more than 9 glucose units, of *dextran 40 IPRS* and of the substance under examination, using the following expression:

$$M_w = \sum w_i \times m_i$$

where, M_w = average molecular mass of the dextran;
m_i = molecular mass of oligosaccharide *i*;
w_i = weight proportion of oligosaccharide *i*.

Use the following m_i values for the calculation:

Oligosaccharide *i*	m_i
glucose	180
isomaltose	342
isomaltotriose	504
isomaltotetraose	666
isomaltopentaose	828
isomaltohexaose	990
isomaltoheptaose	1152
isomaltooctaose	1314
isomaltononaose	1476
isomaltodecaose	1638
isomaltoundecaose	1800
isomaltododecaose	1962
isomaltotridecaose	2124
isomaltotetradecaose	2286
isomaltopentadecaose	2448
isomaltohexadecaose	2610
isomaltoheptadecaose	2772
isomaltooctadecaose	2934
isomaltononadecaose	3096

Inject reference solution (a). The test is not valid unless the values obtained for *dextran 40 IPRS* are within the values stated on the label.

The average molecular mass is 35,000 to 45,000. The average molecular mass of the 10 per cent high fraction is not more than 1,10,000. The average molecular mass of the 10 per cent low fraction is not less than 7,000.

Nitrogen-containing substances. Not more than 110 ppm of N.

Determine the content of nitrogen, Method A (2.3.30),using 0.2 g and heating for 2 hours. Collect the distillate in a mixture of 0.5 ml of *bromocresol green solution*, 0.5 ml of *methyl red solution* and 20 ml of *water*. Titrate with *0.01 M hydrochloric acid*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

Residual solvents (5.4). Not more than 0.5 per cent *ethanol*, 0.05 per cent *methanol* and sum of solvents other than *ethanol*, *methanol* and *propanol* is not more than 0.5 per cent calculated as *propanol*.

Sulphated ash (2.3.18). Not more than 0.3 per cent,determined on 0.5 g.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 1.0 g by drying in an oven at 105° for 5 hours.

Bacterial endotoxins (2.2.3). Not more than 10 Endotoxin Units per g of dextran 40.

Microbial contamination (2.2.9).Total aerobic viable count is not more than 100 CFU per g.

Dextran 40 Infusion

Dextran 40 Injection; Dextran 40 Intravenous Infusion

Dextran 40 Infusion is a sterile solution containing Dextran 40 in Dextrose Injection or in Sodium Chloride Injection.

Dextran 40 Infusion contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextran 40.

Description. An almost colourless, slightly viscous solution.

Tests

Acidity. Titrate 25.0 ml of infusion with *0.01 M sodium hydroxide* using *phenol red solution* as indicator. Not more than 2.0 ml of *0.01 M sodium hydroxide* is required to neutralise the solution.

Molecular size. For solutions in Dextrose Injection, before proceeding with tests A, B and C add 4 volumes of *ethanol (95 per cent)*, centrifuge and dissolve the residue in a volume of Sodium Chloride Injection sufficient to restore the original volume.

A. Determine the viscosity (2.4.28) ratios by Method A, using size C U-tube viscometer at 37°, of solutions in *saline solution* containing about 3.5, 2.5, 1.5 and 0.75 per cent w/v of dextrans,

accurately determined. Calculate the viscosity ratio by dividing the time taken for the meniscus to fall from E to F using the liquid being examined by the time taken using *saline solution*. For each solution, plot (viscosity ratio – 1.00)/concentration (in per cent w/v) against concentration (in per cent w/v). The intercept on the viscosity axis of the straight line joining the points represents the intrinsic viscosity. The intrinsic viscosity is 0.16 to 0.20.

B. Place in each of five stoppered flasks 100 ml of a solution in *saline solution* containing 6 per cent w/v of dextrans and add slowly, with continuous stirring, sufficient *ethanol* to produce a faint cloudiness (about 45 ml is usually required). Add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of *ethanol* to the separate flasks, stopper the flasks and immerse in a water-bath at about 35° with occasional shaking until clear solutions are obtained. Transfer the flasks to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed.

Reject the supernatant liquids, dissolve separately the syrupy residues in sufficient *saline solution* to produce 25.0 ml, remove the ethanol by evaporation at a pressure of about 2 kPa, dilute to 25.0 ml with *water* and determine the optical rotation (2.4.22). From the optical rotations calculate the amount of dextrans precipitated as described under Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine its intrinsic viscosity by the method described under test A; the intrinsic viscosity is not more than 0.27.

C. Place in each of four stoppered flasks 100 ml of a solution in *saline solution* containing 6 per cent w/v of dextrans and add slowly, with continuous stirring, 80, 90, 100 and 110 ml respectively of *ethanol*. Stopper the flasks, transfer to a water-bath maintained at 25.0° ± 0.1° and allow standing overnight or until two clear liquid phases are formed. Separate the supernatant solution from the syrupy residues. Remove the *ethanol* from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against *water* to remove sodium chloride, adjust the volume to 25.0 ml with *water*, add sufficient *sodium chloride* to produce solutions containing 0.9 per cent w/v and determine the optical rotation (2.4.22). From the optical rotations, calculate the amounts of dextrans present as described under Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine the intrinsic viscosity by the method in test A above; the intrinsic viscosity is not less than 0.08.

Content of dextrose *(if present)*. 4.5 per cent to 5.5 per cent w/v.

Dilute 15.0 ml of infusion to 50.0 ml with *water*. To 5.0 ml in a stoppered flask, add 25 ml of a buffer solution containing 14.3 per cent w/v of *sodium carbonate* and 4.0 per cent w/v of *potassium iodide* and 25.0 ml of *0.05 M iodine*. Stopper the flask and allow to stand for exactly 30 minutes at 20°, add 35 ml of *2 M hydrochloric acid* and titrate immediately with *0.1 M sodium thiosulphate*. Repeat the operation using 5 ml of *water*, beginning at the words "add 25 ml of a buffer solution...". The difference between the titrations represents the amount of the iodine required to oxidise the dextrose.

1 ml of *0.05 M iodine* is equivalent to 0.00901 g of dextrose.

Content of sodium chloride *(if present)*. 0.81 per cent to 0.99 per cent w/v.

To a measured volume containing 0.09 g of *sodium chloride*, titrate with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Bacterial endotoxins (2.2.3). Not more than 1.25 Endotoxin Units per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Infusions).

Assay. *For solutions in Dextrose Injection* — Add 0.05 ml of *5 M ammonia* to the required volume and measure the optical rotation (2.4.22). Calculate the content of dextrans from the following expression,

$$0.5076(\alpha - 0.528D)$$

where α is the observed angular rotation and D the content of dextrose per cent w/v, determined in the test for Content of dextrose.

For solutions in Sodium Chloride Injection — Measure the optical rotation (2.4.22), and multiply the value obtained, by 0.5076.

Storage. Store at a temperature not exceeding 30°. The injection should not be exposed to undue fluctuations of temperature.

Labelling. The label states (1) the strength as the per cent w/v of dextrans; (2) the name of the solvent; (3) that the injection should not be used if it is cloudy or if a deposit is present.

Dextran 70

Dextran 70 is a mixture of polysaccharides, principally of the α-1, 6-glucan type. Average relative molecular mass is about 70,000. It is obtained by hydrolysis and fractionation of dextran produced by fermentation of sucrose using strain of *Leuconostoc mesenteroides*.

Category. Plasma substitute.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextran 70 IPRS* or with the reference spectrum of dextran.

B. Specific optical rotation (see Test).

C. Molecular mass distribution (see Test) the peaks in the chromatogram obtained with the test solution corresponds to peaks in the chromatogram obtained with reference solution.

Tests

Solution A. Dissolve 5 g in *water*, heating on a water-bath and dilute to 50.0 ml with *water*.

Appearance of solution. Solution A is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A, add 0.1 ml of *phenolphthalein solution*, the solution remains colourless. Add 0.2 ml of *0.01 M sodium hydroxide*, the solution is pink. Add 0.4 ml of *0.01 M hydrochloric acid*, the solution is colourless, add 0.1 ml of *methyl red solution*, the solution is red or orange.

Specific optical rotation (2.4.22). + 195.0° to + 201.0°, determined on 2.0 per cent w/v solution.

Molecular-mass distribution. Determine by size-exclusion chromatography (2.4.16).

Test solution. Dissolve 6 mg of the substance under examination in 1.0 ml of the mobile phase.

Reference solution (a). Dissolve 6mg of *dextran 70 IPRS* in 1.0 ml of the mobile phase.

Reference solution (b). A solution containing 0.045 per cent w/v of isomaltotriose (3 glucose units), isomaltononaose (9 glucose units) and 0.060 per cent w/v of sodium chloride in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 10 mm, dextran covalently bound to highly cross-linked porous agarose beads, allowing resolution of oligosaccharides in the molecular mass range of 180 to 3000 (two columns coupled in series),
- temperature: 20-25°,
- mobile phase: a 0.292 per cent w/v solution of *sodium chloride*,
- flow rate: 0.07-0.08 ml per minute,
- differential refractometer,
- injection volume: 100 µl.

Identification of peaks, use the chromatogram obtained with reference solution (b) to identify the peaks due to isomaltotriose, isomaltononaose and sodium chloride.

Determine the peak areas. Disregard any peak due to sodium chloride. Calculate the average relative molecular mass M_w and the amount of the fraction with less than 3 and more than 9 glucose units, of *dextran 70 IPRS* and of the substance under examination, using the following expression:

$$M_w = \sum w_i \times m_i$$

where, M_w = average molecular mass of the dextran 1;
m_i = molecular mass of oligosaccharide i;
w_i = weight proportion of oligosaccharide i.

Use the following m_i values for the calculation:

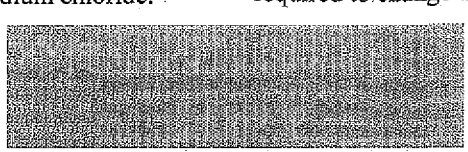
Oligosaccharide i	m_i
glucose	180
isomaltose	342
isomaltotriose	504
isomaltotetraose	666
isomaltopentaose	828
isomaltohexaose	990
isomaltoheptaose	1152
isomaltooctaose	1314
isomaltononaose	1476
isomaltodecaose	1638
isomaltoundecaose	1800
isomaltododecaose	1962
isomaltotridecaose	2124
isomaltotetradecaose	2286
isomaltopentadecaose	2448
isomaltohexadecaose	2610
isomaltoheptadecaose	2772
isomaltooctadecaose	2934
isomaltononadecaose	3096

Inject reference solution (a). The test is not valid unless the values obtained for *dextran 70 IPRS* are within the values stated on the label.

The average molecular mass is 64,000 to76,000. The average molecular mass of the 10 per cent high fraction is not more than 1,85,000. The average molecular mass of the 10 per cent low fraction is not less than 15,000.

Nitrogen-containing substances. Not more than 110 ppm of N.

Determine the content of nitrogen, Method A (2.3.30),using 0.2 g and heating for 2 hours. Collect the distillate in a mixture of 0.5 ml of *bromocresol green solution*, 0.5 ml of *methyl red solution* and 20 ml of *water*. Titrate with *0.01 M hydrochloric acid*. Not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.



Residual solvents (5.4). Not more than 0.5 per cent *ethanol*, 0.05 per cent *methanol* and sum of solvents other than *ethanol*, *methanol* and *propanol* is not more than 0.5 per cent calculated as *propanol*.

Sulphated ash (2.3.18). Not more than 0.3 per cent,determined on 0.5 g.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 1.0 g by drying in an oven at 105° for 5 hours.

Bacterial endotoxins (2.2.3). Not more than 16 Endotoxin Units per g of dextran70.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 100 CFU per g.

Dextran 70 Infusion

Dextran 70 Injection; Dextran 70 Intravenous Infusion

Dextran 70 Infusion is a sterile solution containing Dextran 70 in Dextrose Injection or in Sodium Chloride Injection.

Dextran 70 Infusion contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextran 70.

Description. An almost colourless, slightly viscous solution.

Tests

Acidity. Titrate 25.0 ml of infusion with *0.01 M sodium hydroxide* using *phenol red solution* as indicator. Not more than 1.25 ml of *0.01 M sodium hydroxide* is required to neutralise the solution.

Molecular size. For solutions in Dextrose Injection, before proceeding with tests A, B and C add 4 volumes of *ethanol (95 per cent)*, centrifuge and dissolve the residue in a volume of Sodium Chloride Injection sufficient to restore the original volume.

A. Determine the viscosity (2.4.28) ratios by Method A, using size C U-tube viscometer at 37°, of solutions in *saline solution* containing about 3.5, 2.5, 1.5 and 0.75 per cent w/v of dextrans, accurately determined. Calculate the viscosity ratio by dividing the time taken for the meniscus to fall from E to F using the liquid being examined by the time taken using *saline solution*.For each solution, plot (viscosity ratio – 1.00)/ concentration (in per cent w/v) against concentration (in per cent w/v). The intercept on the viscosity axis of the straight line joining the points represents the intrinsic viscosity.The intrinsic viscosity is 0.22 to 0.27.

B. Place in each of five stoppered flasks 100 ml of a solution in *saline solution* containing 6 per cent w/v of dextrans and add slowly, with continuous stirring, sufficient *ethanol* to produce a faint cloudiness (about 45 ml is usually required). Add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of *ethanol* to the separate flasks, stopper the flasks and immerse in a water-bath at about 35° with occasional shaking until clear solutions are obtained. Transfer the flasks to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed.

Reject the supernatant liquids, dissolve separately the syrupy residues in sufficient *saline solution* to produce 25.0 ml, remove the ethanol by evaporation at a pressure of about 2 kPa, dilute to 25.0 mlwith *water* and determine the optical rotation (2.4.22). From the optical rotations calculate the amount of dextrans precipitated as described under Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine its intrinsic viscosity by the method described under test A; the intrinsic viscosity is not more than 0.36.

C. Place in each of four stoppered flasks 100 ml of a solution in *saline solution* containing 6 per cent w/v of dextrans and add slowly, with continuous stirring, 80, 90, 100 and 110 ml respectively of *ethanol*. Stopper the flasks, transfer to a water 'bath maintained at 25.0° ± 0.1° and allow standing overnight or until two clear liquid phases are formed. Separate the supernatant solution from the syrupy residues. Remove the *ethanol* from each supernatant solution separately by evaporation at a pressure of 2 kPa, dialyse in cellophane tubing against *water* to remove sodium chloride, adjust the volume to 25.0 ml with *water*, add sufficient *sodium chloride* to produce solutions containing 0.9 per cent w/v and determine the optical rotation (2.4.22). From the optical rotations, calculate the amounts of dextrans present as described under Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine the intrinsic viscosity by the method in test A above; the intrinsic viscosity is not less than 0.13.

Content of dextrose *(if present)*. 4.5 per cent to 5.5 per cent w/v.

Dilute 15.0 ml of infusion to 50.0 ml with *water*. To 5.0 ml in a stoppered flask, add 25 ml of a buffer solution containing 14.3 per cent w/v of *sodium carbonate* and 4.0 per cent w/v of *potassium iodide* and 25.0 ml of *0.05 M iodine*. Stopper the flask and allow to stand for exactly 30 minutes at 20°, add 35 ml of *2 M hydrochloric acid* and titrate immediately with *0.1 M sodium thiosulphate*. Repeat the operation using 5 ml of *water*, beginning at the words "add 25 ml of a buffer solution...". The difference between the titrations represents the amount of the iodine required to oxidise the dextrose.

1 ml of *0.05 M iodine* is equivalent to a 0.00901 g of dextrose.

Content of sodium chloride *(if present)*. 0.81 per cent to 0.99 per cent w/v.

To a measured volume containing 0.09 g of *sodium chloride*, titrate with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Bacterial endotoxins (2.2.3). Not more than 1.21 Endotoxin Units per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Infusions).

Assay. *For solutions in Dextrose Injection* — Add 0.05 ml of *5 M ammonia* to the required volume and measure the optical rotation (2.4.22). Calculate the content of dextrans from the following expression,

$$0.5076(\alpha - 0.528D)$$

where α is the observed angular rotation and D the content of dextrose per cent w/v, determined in the test for Content of dextrose.

For solutions in Sodium Chloride Injection — Measure the optical rotation (2.4.22), and multiply the value obtained, by 0.5076.

Storage. Store at a temperature not exceeding 30°. The injection should not be exposed to undue fluctuations of temperature.

Labelling. The label states (1) the strength as the per cent w/v of dextrans; (2) the name of the solvent; (3) that the injection should not be used if it is cloudy or if a deposit is present.

Dextran 110 Injection

Dextran 110 Intravenous Infusion

Dextran 110 Injection is a sterile solution, in Dextrose Injection or in Sodium Chloride Injection, of dextrans of average molecular weight of about 110,000, derived from the dextrans produced by the fermentation of sucrose by means of a certain strain of *Leuconostoc mesenteroides*. The dextrans are polymers of dextrose in which the linkages between the dextrose units are almost entirely of the α-1→6 type.

Dextran 110 Injection contains not less than 5.5 per cent and not more than 6.5 per cent w/v of dextrans.

Category. Plasma substitute.

Description. An almost colourless, slightly viscous solution.

Tests

pH (2.4.24). 3.5 to 6.5 for solutions in Dextrose Injection; 5.0 to 7.0 for solutions in Sodium Chloride Injection.

Molecular size. For solutions in Dextrose Injection, before proceeding with tests A and B, add 4 volumes of *ethanol (95 per cent)*, centrifuge and dissolve the residue in a volume of *Sodium Chloride Injection* sufficient to restore the original volume.

A. Determine the viscosity (2.4.28) ratios by Method A, using size C U-tube viscometer at 37°, of solutions in *saline solution* containing about 2.0, 1.0, 0.5 and 0.25 per cent w/v of dextrans, accurately determined. For each solution, plot (viscosity ratio - 1.00)/concentration (in per cent w/v) against concentration (in per cent w/v). The intercept on the viscosity ratio axis of the straight line joining the points represents the intrinsic viscosity; the intrinsic viscosity is 0.27 to 0.32.

B. Place 100 ml in each of five stoppered flasks and adjust the temperature to 25.0° ± 0.1°. With precautions to maintain this temperature, add slowly with continuous stirring sufficient *ethanol* to produce a faint cloudiness (about 45 ml is usually required). Add 0.5, 1.0, 1.5, 2.0 and 2.5 ml of *ethanol* to the separate flasks, stopper the flasks and immerse in a water-bath at about 35° with occasional shaking until clear solutions are obtained. Transfer the flasks to a water-bath maintained at 25.0° ± 0.1° and allow to stand overnight or until two clear liquid phases are formed. Reject the supernatant liquids, dissolve separately the syrupy residues in sufficient *saline solution* to produce 25.0 ml, remove the *ethanol* by evaporation at a pressure of about 2 kPa, dilute to 25.0 ml with *water* and determine the optical rotation (2.4.22). From the optical rotations calculate the amount of dextrans precipitated as described under Assay. Choose that fraction containing as nearly as possible but not more than 10 per cent of the dextrans present in the injection and determine its intrinsic viscosity by the method described under test A; the intrinsic viscosity is not more than 0.40.

Content of dextrose. For solutions in Dextrose Injection, between 4.5 and 5.5 per cent w/v determined by the following method. Dilute 15.0 ml to 50.0 ml with *water*. To 5.0 ml in a stoppered flask add 25 ml of a buffer solution containing 14.3 per cent *w/v of sodium carbonate* and 4.0 per cent *w/v* of *potassium iodide* and 25.0 ml of *0.05 M iodine*. Stopper the flask and allow to stand for exactly 30 minutes at 20°, add 30 ml of *dilute hydrochloric acid* and titrate immediately with *0.1 M sodium thiosulphate*. Repeat the operation beginning at the words "add 25 ml of a buffer solution..." but using 5 ml of *water* in place of 5 ml of the preparation under examination. The difference between the titrations represents the amount of iodine required to oxidise the dextrose.

1 ml of *0.05 M iodine* is equivalent to a 0.00901 g of dextrose.

Acetone. To 10 ml add sufficient *ammonium sulphate* to give a saturated solution, add 1 ml of *sodium nitroprusside solution* and 5 ml of *strong ammonia solution*, and allow to stand for 10 minutes. Any purple colour produced is not more intense than that produced by treating in the same manner 10 ml of a 0.02 per cent v/v solution of *acetone*.

Content of sodium chloride (*if present*). For solutions in Sodium Chloride Injection, 0.81 to 0.99 per cent w/v, determined by the following method. Titrate a measured volume containing 0.1 g of sodium chloride with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Ethanol. Distil 100 ml, collect the first 45 ml of distillate and dilute to 50 ml with *water*. Mix 10 ml of *0.0167 M potassium dichromate* and 10 ml of *sulphuric acid* in a stoppered boiling tube, immediately add 5 ml of the distillate, mix, stopper the tube, and allow to stand for 5 minutes. Transfer to a 500-ml flask, dilute to about 300 ml with *carbon dioxide-free water*, add 2 g of *potassium iodide* and 1 ml of a 10 per cent w/v solution of *potassium thiocyanate*, allow to stand for 5 minutes and titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the determination beginning at the words "Mix 10 ml of *0.0167 M potassium dichromate*.." but using 5 ml of *water* in place of 5 ml of the distillate. The difference between the titrations is not more than 4.2 ml.

Heavy metals (2.3.13). To 4.0 ml add 5 ml of *dilute acetic acid* and sufficient *water* to produce 25.0 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

Nitrogen (2.3.30). Determine by Method B, using 50 ml. For solutions in Dextrose Injection, use 30 ml of *nitrogen-free sulphuric acid*. For solutions in Sodium Chloride Injection use 20 ml of *nitrogen-free sulphuric acid*.

Not more than 0.35 ml of *0.05 M sulphuric acid* is required.

Sulphated ash. Titrate 25 ml with *0.1 M silver nitrate* using *potassium chromate solution* as indicator. Deduct the theoretical value of the sulphated ash due to the sodium chloride present.

1 ml of *0.1 M silver nitrate* is equivalent to 0.007102 g of sulphated ash (0.05 per cent w/v).

Foreign protein. Inject 0.5 ml on three occasions at intervals of 2 days into the peritoneal cavity of each of six healthy guinea-pigs weighing not less than 250 g that have not previously been treated with any material that will interfere with the test. Inject 0.2 ml intravenously into each of the three guinea-pigs 14 days after the first intra-peritoneal injection, and into each of the other three guinea-pigs 21 days after the first intra-peritoneal injection. Observe the guinea-pigs for 30 minutes after each intravenous injection and again 24 hours later; the animals exhibit no signs of anaphylaxis such as coughing, bristling of hair or respiratory distress.

Bacterial endotoxins (2.2.3). Not more than 1.25 Endotoxin Units per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Infusions).

Assay. *For solutions in Dextrose Injection* — Add a drop of *dilute ammonia solution* to the required volume and determine the optical rotation (2.4.22). Calculate the content of dextrans from the following expression $0.5076(\alpha - 0.528D)$, where α is the observed angular rotation and D the content of dextrose per cent w/v, determined in the test for Content of dextrose.

For solutions in Sodium Chloride Injection — Measure the optical rotation (2.4.22), and multiply the value obtained by 0.5076.

Storage. Store at a temperature not exceeding 30°. The injection should not be exposed to undue fluctuations of temperature.

Labelling. The label states (1) the strength as the percentage w/v of dextrans; (2) the name of the solvent; (3) the strain of *Leuconostoc mesenteroides* used; (4) that the injection should not be used if it is cloudy or if a deposit is present.

Dextrin

Dextrin is starch partially hydrolysed by heat with or without the aid of suitable acids and buffers.

Category. Pharmaceutical aid (tablet excipient).

Description. A white or pale yellow powder.

Identification

A. *Microscopic characteristics* — Granules have similar appearance to the starch from which the dextrin has been prepared. In dextrin prepared from maize starch many of the granules show concentric striations and in dextrin prepared from potato starch concentric striations are not clearly visible; the hilum may be bicleft and some of the granules may be distorted.

B. Boil 1 g in 50 ml of *water*, cool. To 5 ml of the cloudy suspension, add a drop of *iodine solution* and mix; a purple colour is produced.

C. To 5 ml of the suspension produced in test B add 2 ml of *2 M sodium hydroxide*, mix, add dropwise with shaking 0.5 ml of *cupric sulphate solution* and boil; a red precipitate is produced.

Tests

Acidity. Add 10 g to 100 ml of *ethanol (70 per cent)*, previously neutralised to *phenolphthalein solution*, shake for 1 hour, filter and titrate 50 ml of the filtrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator. Not more than 1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method A (40 ppm).

Chlorides (2.3.12). Dissolve 2.5 g in 50 ml of boiling *water*, cool, dilute to 100 ml with *water* and filter. 5 ml of the filtrate diluted to 15 ml complies with the limit test for chlorides (0.2 per cent).

Ethanol-soluble substances. Not more than 1 per cent, determined by the following method. Boil under a reflux condenser 1 g with 20 ml of *ethanol (95 per cent)* for 5 minutes and filter while hot. Evaporate 10 ml of the filtrate on a water-bath, dry the residue at 105° and weigh.

Protein. Not more than 0.5 per cent, determined by the following method. Carry out Method A for the determination of nitrogen (2.3.30), using 5 g, weighed, and 30 ml of *nitrogen-free sulphuric acid*. Calculate the content of protein by multiplying the percentage of nitrogen in the substance under examination by 6.25.

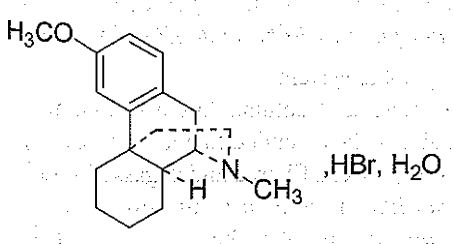
Reducing substances. Not more than 10 per cent, calculated as dextrose, $C_6H_{12}O_6$, determined by the following method. Weigh a quantity containing 2 g of the dried substance, add 100 ml of *water*, shake for 30 minutes, dilute to 200.0 ml with *water* and filter. To 10 ml of *cupri-tartaric solution* add 20.0 ml of the filtrate, mix and heat at a rate such that the solution is brought to boil in 3 minutes. Boil for a further 2 minutes and cool quickly. Add 5 ml of a 30 per cent w/v solution of *potassium iodide* and 10 ml of *1 M sulphuric acid*, mix and titrate immediately with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the procedure using 20.0 ml of a 0.1 per cent w/v solution of *dextrose* in place of the filtrate beginning at the words "To 10 ml of...". Carry out a blank titration using 20 ml of *water* in place of 20.0 ml of the sample filtrate. The titre obtained with the sample filtrate is not greater than the titre obtained with the dextrose solution.

Ash (2.3.19). Not more than 1 per cent.

Loss on drying (2.4.19). Not more than 12 per cent, determined on 1.0 g by drying in an oven at 110°.

Storage. Store protected from moisture.

Dextromethorphan Hydrobromide



$C_{18}H_{25}NO,HBr,H_2O$ Mol. Wt. 370.3

Dextromethorphan Hydrobromide is *ent*-3-methoxy-9a-methylmorphinan hydrobromide monohydrate.

Dextromethorphan Hydrobromide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{25}NO,HBr$ calculated on the anhydrous basis.

Category. Cough suppressant.

Description. An almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextromethorphan hydrobromide IPRS* or with the reference spectrum of dextromethorphan hydrobromide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at 278 nm.

C. It gives the reaction of bromides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. Dissolve 0.4 g in *carbon dioxide-free water* with gentle heat, cool and dilute to 20 ml with the same solvent. Add 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow and not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour to red.

Specific optical rotation (2.4.22). +28.0° to +30.0°, determined in a 2.0 per cent w/v solution in *0.1 M hydrochloric acid*.

***N,N*-Dimethylaniline**. Dissolve 0.5 g in 20 ml *water* with the help of gentle heat on a water-bath, cool and add 2 ml of *2 M acetic acid*, 1 ml of a 1 per cent w/v solution of *sodium nitrite* and sufficient *water* to produce 25 ml. The resulting solution is not more intensely coloured than that obtained by treating at the same time and in the same manner a solution containing 5 µg of *N,N-dimethylaniline* in 20 ml of *water*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve 2 mg of *dextromethorphan impurity A IPRS* in 2 ml of the test solution and dilute to 25.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 3.11 g of *docusate sodium* in a mixture of 400 ml of *water* and 600 ml of *acetonitrile*. Add 0.56 g of *sodium nitrate*, adjusted to pH 2.0 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Dextromethorphan impurity B[1]	0.4	—
Dextromethorphan impurity C[2]	0.8	0.2
Dextromethorphan impurity D[3]	0.9	—
Dextromethorphan (Retention time is about 22 minutes)	1.0	—
Dextromethorphan impurity A[4]	1.1	—

[1]*ent*-17-methylmorphinan-3-ol,

[2]*ent*-3-methoxy-17-methylmorphinan-10-one,

[3]*ent*-(14*S*)-3-methoxy-17-methylmorphinan,

[4]*ent*-3-methoxymorphinan.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to dextromethorphan and dextromethorphan impurity A is not less than 1.5.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak due to dextromethorphan impurities A, B, C and D, each of, is not more than the area of the principal peak in the chromatogram obtained with reference solution (0.5 per cent) and the area of not more than one such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 4.0 to 5.5 per cent, determined on 0.2 g.

Assay. Dissolve 0.3 g in 20 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03523 g of $C_{18}H_{25}NO,HBr$.

Storage. Store protected from light.

Dextromethorphan Hydrobromide Syrup

Dextromethorphan Hydrobromide Syrup is a solution of Dextromethorphan Hydrobromide in a suitable flavoured vehicle.

Dextromethorphan Hydrobromide Syrup contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dextromethorphan hydrobromide, $C_{18}H_{25}NO,HBr,H_2O$.

Usual strength. 13.5 mg in 5 ml.

Identification

A. To 50 ml, add 20 ml of *water*, 5 ml of *2.5 M sodium hydroxide* and extract with three quantities, each of 40 ml of *hexane*, collect the *hexane layer* and filter through *anhydrous sodium sulphate* placed over absorbent cotton wetted with *hexane*. Evaporate the combined extracts at 50° under nitrogen to dryness, dissolve and dilute the residue in 10 ml of *chloroform*; the solution is dextrorotatory (2.4.22). Retain the chloroform solution for test B.

B. Evaporate the chloroform solution obtained from test A on a water-bath to dryness, dissolve the residue in 2 ml of *1 M sulphuric acid* and add 1 ml of a solution prepared freshly by dissolving 700 mg of *mercuric nitrate* in 4 ml of *water*, adding 100 mg of *sodium nitrate*, mixing and filtering; the solution gives no colour, but after heating, a yellow to red colour develops in about 15 minutes.

Tests

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the syrup containing about 10 mg of Dextromethorphan Hydrobromide to 100.0 ml with *water*.

Reference solution. A 0.01 per cent w/v solution of *dextromethorphan hydrobromide IPRS* in *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed solution of *0.007 M sodium nitrate* in a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*, adjusted to pH 3.4 with *glacial acetic acid*,

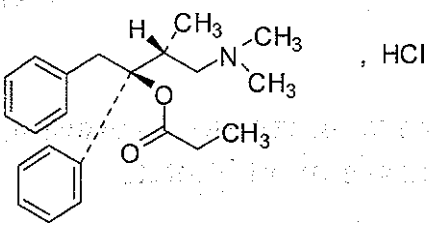
– flow rate: 1 ml per minute,
– spectrophotometer set at 280 nm,
– injection volume: 20 µl.

Calculate the content of $C_{18}H_{25}NO,HBr,H_2O$ in the syrup.

Storage. Store protected from light.

Dextropropoxyphene Hydrochloride

Propoxyphene Hydrochloride



$C_{22}H_{29}NO_2,HCl$ Mol. Wt. 375.9

Dextropropoxyphene Hydrochloride is (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl propionate hydrochloride.

Dextropropoxyphene Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{22}H_{29}NO_2,HCl$, calculated on the dried basis.

Category. Analgesic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextropropoxyphene hydrochloride IPRS* or with the reference spectrum of dextropropoxyphene hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in *0.01 M hydrochloric acid*, shows three absorption maxima, at 252 nm, 257 nm and 263 nm and two shoulders, at 240 nm and 246 nm. The ratio of the absorbance at the maximum at 257 nm to that at 252 nm is 1.22 to 1.28. The ratio of the absorbance at the maximum at 257 nm to that at 263 nm is 1.29 to 1.35.

C. Solution A gives the reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. Dilute 10 ml of solution A to 25 ml with *carbon dioxide-free water*. To 10 ml of the solution, add 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*, the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*, the solution is red.

Specific optical rotation (2.4.22). + 52° to + 57°, determined in a 1.0 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 10.0 ml of the mobile phase.

Reference solution (a). Dilute 0.5 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 50 mg of the substance under examination in 2.5 ml of *2 M ethanolic potassium hydroxide*. Add 2.5 ml of *water* and boil under a reflux condenser for 30 minutes. Add 2.5 ml of *dilute hydrochloric acid* and dilute to 50.0 ml with the mobile phase.

Chromatographic system
– a stainless steel column 12.5 cm x 4.6 mm, packed with silica gel (5 µm),
– mobile phase: a mixture of 5 volumes of *0.2 M phosphate buffer solution pH 7.5*, 8.4 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 51.6 volumes of 0.09 per cent w/v solution of *cetyltrimethylammonium bromide* in *water*,
– flow rate: 1 ml per minute,
– spectrophotometer set at 220 nm,
– injection volume: 20 µl.

Equilibrate the chromatographic system by passage of the mobile phase for 16 hours.

Inject reference solution (a) and (b). Run the chromatogram twice the retention time of the principal peak. The test is not valid unless the chromatogram obtained with reference solution (a) shows signal-to-noise ratio of the principal peak is not less than 5 and the chromatogram obtained with reference solution (b) shows two peaks with a resolution of not less than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Heavy metals (2.3.13). 12 ml of solution A complies with the limit test for heavy metals, Method D (20 ppm), using 10 ml of *lead standard solution (1 ppm Pb)*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.27 g in 60 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03759 g of $C_{22}H_{30}ClNO_2$.

Storage. Store protected from light.

Dextropropoxyphene Capsules

Dextropropoxyphene Hydrochloride Capsules

Dextropropoxyphene Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dextropropoxyphene, $C_{22}H_{29}NO_2$.

Usual strength. 65 mg.

Identification

Shake a quantity of the content of capsules containing about 0.15 g of Dextropropoxyphene with 5 ml of *chloroform* and filter. The filtrate complies with the following tests.

A. Evaporate 3 ml to dryness and dry the residue at 105° for 1 hour. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextropropoxyphene napsilate IPRS* or with the reference spectrum of dextropropoxyphene napsilate.

B. Evaporate 0.05 ml in a porcelain dish and streak the spot with 5 per cent v/v solution of *formaldehyde* in *sulphuric acid;* a purple colour is produced.

C. Evaporate 0.4 ml on a piece of filter paper and burn the residue by the method for oxygen-flask combustion (2.3.34), using 5 ml of *1.25 M sodium hydroxide* as the absorbing liquid. When the process is complete, dilute the liquid to 25 ml with *water*. To 5 ml of the solution, add 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *1 M hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*. The solution becomes turbid.

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 0.5 g of dextropropoxyphene with 25 ml of *chloroform* and filter through absorbent cotton, washing the flask and filter with small quantities of *chloroform*. Add to the combined filtrates a mixture of 50 ml of *water* and 5 ml of *5 M sodium hydroxide*. Shake, allow the layers to separate and wash the chloroform extract with 25 ml of *water*. Extract the aqueous layer with five 25 ml quantities of *chloroform*, washing each extract with the 25 ml of *water* and adding it to the original extract. Dry the combined extracts with *anhydrous sodium sulphate*, evaporate to about 3 ml on a water-bath in a current of air, remove from the water-bath and allow to evaporate to dryness at room temperature. Titrate with *0.1 M perchloric acid*, using a few drops of *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03395 g of $C_{22}H_{29}NO_2$.

Labelling. The lable states the quantity of active ingredient in terms of the equivalent amount of dextropropoxyphene.

Dextropropoxyphene Hydrochloride and Paracetamol Tablets

Co-proxamol Tablets

Dextropropoxyphene Hydrochloride and Paracetamol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of dextropropoxyphene hydrochloride, $C_{22}H_{29}NO_2$,HCl and paracetamol, $C_8H_9NO_2$.

Usual strength. Paracetamol, 300 mg and Dextropropoxyphene Hydrochloride, 25 mg.

Identification

A. Disperse a quantity of the powdered tablets containing 0.1 g of Dextropropoxyphene Hydrochloride in 20 ml of *0.1M hydrochloric acid*, with the aid of ultrasound for 5 minutes and filter. To the filtrate, add 5 ml of *2 M sodium hydroxide*, extract twice with 25 ml quantities of *dichloromethane*, wash the combined extracts with 10 ml of *water*, shake with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 2 ml of *dichloromethane* and add 50 µl of the solution, drop wise, onto the surface of a disc prepared from about 0.3 g of *potassium bromide*, evaporate the solvent, dry the disc at 50° for 2 minutes. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextropropoxyphene hydrochloride IPRS* treated in the same manner or with the reference spectrum of dextropropoxyphene.

B. Disperse a quantity of the powdered tablets containing 0.325 g of Paracetamol in 10 ml of *acetone*, with the aid of ultrasound for 5 minutes, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *paracetamol IPRS* or with the reference spectrum of paracetamol.

Tests

For Paracetamol —

Dissolution (2.5.2).

Apparatus. No 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 5.8*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium, filter and dilute a suitable volume of the filtrate with *0.1M sodium hydroxide*, if necessary, to obtain a solution containing 0.00075 per cent w/v of paracetamol. Measure the absorbance of the resulting solution at the maximum at about 257 nm (2.4.7). Calculate the content of $C_8H_9NO_2$ taking 715 as the specific absorbance at 257 nm.

Q. Not less than 70 per cent of the stated amount of $C_8H_9NO_2$.

4-Aminophenol. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.5 g of Paracetamol in the mobile phase, with the aid of ultrasound and dilute to 50.0 ml with the mobile phase.

Reference solution. A 0.001 per cent w/v solution of *4-aminophenol IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Nucleosil C18),
- mobile phase: *0.01M sodium butane sulphonate* in a mixture of 85 volumes of *water*, 15 volumes of *methanol* and 0.4 volume of *formic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to 4-aminophenol is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances.

For Dextropropoxyphene Hydrochloride —

Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 25 mg of Dextropropoxyphene Hydrochloride in 5 ml of *acetonitrile* by shaking for 2 minutes, add 5 ml of *water* and further shake for 5 minutes, dilute to 25.0 ml with *water* and filter.

Reference solution. A solution containing 0.0005 per cent w/v, each of, *4-dimethylamino-3-methyl-1, 2-diphenylbutan-2-ol hydrochloride IPRS* and *(1S,2R)-1-benzyl-3-dimethyl-amino-2-methyl-1-phenylpropyl acetate IPRS* in a mixture of 1 volume of *acetonitrile* and 4 volumes of *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *0.2M sodium perchlorate*, previously adjusted to pH 2.0 with *7M hydrochloric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to 4-dimethylamino-3-methyl-1, 2-diphenylbutan-2-ol hydrochloride and (1*S*, 2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate is not less than 1.5.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peaks corresponding to 4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride and (1*S*, 2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate, each of, is not more than the area of the corresponding peaks in the chromatogram obtained with the reference solution (0.5 per cent).

For Paracetamol —

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *toluene*, 25 volumes of *acetone* and 65 volumes of *chloroform*.

Test solution (a). Disperse a quantity of powdered tablets containing 1.0 g of Paracetamol in 5 ml of *peroxide free ether*, shake for 30 minutes, centrifuge at 1000 revolution per minute for 15 minutes or until a clear supernatant liquid is obtained and use the supernatant liquid.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *ethanol (95 per cent)*.

Reference solution (a). A 0.005 per cent w/v solution of *4-chloroacetanilide IPRS* in *ethanol (95 per cent)*.

Reference solution (b). A solution containing 0.25 per cent w/v of *4-chloroacetanilide IPRS* and 0.1 per cent w/v of *paracetamol IPRS* in 100.0 ml *ethanol (95 per cent)*.

Apply to the plate 200 µl of test solution (a) and 40 µl, each of test solution (b), reference solution (a) and reference solution (b). Allow the mobile phase to rise 14 cm. Dry the plate in hot air and examine under ultraviolet light at 254 nm. In the chromatogram obtained with test solution (a), any spot corresponding to 4-chloroacetanilide is not more intense than the principal spot in the chromatogram obtained with the

reference solution (a) (0.005 per cent). In the chromatogram obtained with test solution (b), any secondary spot with an Rf value lower than that of 4-chloroacetanilide is not more intense than the principal spot in the chromatogram obtained with the reference solution (b) (0.25 per cent). The test is not valid unless two clearly separated spots are seen in the chromatogram obtained with reference solution (b), the spot corresponding to 4-chloroacetanilide having the higher Rf value.

Other tests. Comply with the tests stated under Tablets

Assay.

For Dextropropoxyphene Hydrochloride —

Determine by liquid chromatography (2.4.14)

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 32.5 mg of Dextroprpoxyphene Hydrochloride in 100 ml of *0.02 M hydrochloric acid*, with aid of ultrasound for 15 minutes, dilute to 500.0 ml with a mixture of equal volume of *acetonitrile* and *0.02 M hydrochloric acid* and filter.

Reference solution (a). A 0.0065 per cent w/v solution of *dextropropoxyphene hydrochloride IPRS* in a mixture of 40 volumes of *acetonitrile* and 60 volumes of *0.02 M hydrochloric acid.*

Reference solution (b). A solution containing 0.0005 per cent w/v solution, each of, *4-dimethylamino-3-methyl-1, 2-diphenylbutan-2-ol hydrochloride IPRS* and *(1S, 2R)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate IPRS* in a mixture of 1 volume of *acetonitrile* and 4 volumes of *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *0.2 M sodium perchlorate*, previously adjusted to pH 2.0 with *7 M hydrochloric acid.*
- flow rate: 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to 4-dimethylamino-3-methyl-1, 2-diphenylbutan-2-ol hydrochloride and *(1S, 2R)*-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate is not more than 1.5

Inject reference solution (a) and the test solution.

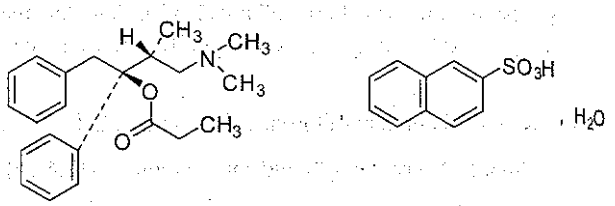
Calculate the content of $C_{22}H_{29}NO_2$.HCl in the tablets.

For Paracetamol —

Disperse a quantity of powdered tablets containing 325 mg of Paracetamol in 5 ml of *water*, add 100 ml of *methanol* and shake. Add 300 ml of *water*, shake for 5 minutes and dilute to 500.0 ml with *water*, mix and filter. Dilute 5.0 ml of the filtrate to 250.0 ml with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 257 nm (2.4.7). Calculate the content of $C_8H_9NO_2$ in the tablets taking 715 as the specific absorbance at 257 nm.

Storage. Store protected from light.

Dextropropoxyphene Napsilate



$C_{22}H_{29}NO_2,C_{10}H_8O_3S,H_2O$ Mol. Wt. 565.8

Dextropropoxyphene Napsylate is (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl propionate naphthalene-2-sulphonate monohydrate.

Dextropropoxyphene Napsilate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{22}H_{29}NO_2,C_{10}H_8O_3S$, calculated on the anhydrous basis.

Category. Analgesic.

Description. A white powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dextropropoxyphene napsilate IPRS* or with the reference spectrum of dextropropoxyphene napsilate.

B. Dissolve 25 mg in 5 ml of *dichloromethane*, evaporate 0.05 ml of the solution in a porcelain dish and streak the spot with 5 per cent v/v solution of *formaldehyde solution* in *sulphuric acid*; a purple colour is produced.

C. Determine by the oxygen-flask method (2.3.34), burning 0.02 g, using 5 ml of *1.25 M sodium hydroxide* as the absorbing liquid. When the process is complete, dilute the liquid to 25 ml with *water*. To 5 ml of the solution so obtained add 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *1 M hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*. The solution becomes turbid.

Tests

Specific optical rotation (2.4.22). +26.0° to +31.0°, determined in a 5.0 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by gas chromatography (2.4.13).

Solution A. Dissolve 10 mg of *triphenylamine* (internal standard) in 50 ml of *dichloromethane*.

Test solution (a). Dissolve 0.3 g of the substance under examination in 5 ml of *dichloromethane*, add 10 ml of *water*, 2 ml of *1.25 M sodium hydroxide* and 15 ml of *dichloromethane* and shake. Extract the aqueous layer with two 20 ml quantities of *dichloromethane*. Shake the combined dichloromethane extracts with 5 g of *anhydrous sodium sulphate*, filter and evaporate to dryness at a temperature not exceeding 40° using a rotary evaporator. Dissolve the residue in 10 ml of *dichloromethane*.

Test solution (b). Prepare in the same manner as test solution (a) but add 5 ml of solution A to the initial solution of the substance under examination.

Reference solution. Add 5 ml of solution A, 10 ml of *water*, 2 ml of *1.25 M sodium hydroxide* and 15 ml of *dichloromethane* to 5 ml of a solution in *dichloromethane* containing 0.022 per cent w/v of *(1S,2R)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate IPRS* and 0.02 per cent w/v of *4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride IPRS* and shake. Extract the aqueous layer with two 20 ml quantities of *dichloromethane*. Shake the combined dichloromethane extracts with 5 g of *anhydrous sodium sulphate*, filter and evaporate to dryness at a temperature not exceeding 40° using a rotary evaporator. Dissolve the residue in 10 ml of *dichloromethane*.

Chromatographic system

- a glass column 0.6 m x 3 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of dimethyl silicone fluid,
- temperature:
  column 160°,
  inlet port and detector at 150°,
- flow rate: 60 ml per minute, using nitrogen as the carrier gas.

The peaks, other than the solvent peak, in the chromatogram obtained with the reference solution are due, in order of emergence, to (a) the internal standard, (b) (1*S*,2*R*)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl acetate and (c) 4-dimethylamino-3-methyl-1,2-diphenylbutan-2-ol hydrochloride. In the chromatogram obtained with test solution (b) the ratio of the area of any peak corresponding to (b) to that of the peak due to (a) and the ratio of the area of any peak corresponding to (c) to that of the peak due to (a) are not more than the corresponding ratios in the chromatogram obtained with reference solution (0.67 per cent each).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

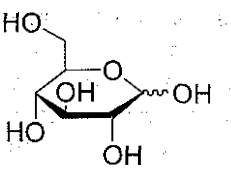
Water (2.3.43). 3.0 to 5.0 per cent, determined on 0.5 g.

Assay. Weigh 0.75 g add 50 ml of *water*, swirl to disperse, add 5 ml of *5 M sodium hydroxide* and extract with five 25 ml quantities of *dichloromethane*, washing each extract with the same 20 ml of *water*. Dry the combined extracts with *anhydrous sodium sulphate*, evaporate on water-bath in a current of air and allow to evaporate to dryness at room temperature. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05478 g of $C_{22}H_{29}NO_2,C_{10}H_8O_3S$.

Dextrose

Glucose; D-Glucose



$C_6H_{12}O_6$ Mol. Wt. 180.2 (anhydrous)

$C_6H_{12}O_6,H_2O$ Mol. Wt. 198.2 (monohydrate)

Dextrose is D-(+)-glucopyranose or D-(+)-glucopyranose monohydrate.

Category. Nutrient; fluid replenisher.

Description. A white crystalline powder.

Identification

A. When heated, it melts, swells up and burns, and an odour of burnt sugar is perceptible.

B. Dissolve 0.1 g in 10 ml of *water*, add 3 ml of *potassium cupri-tartrate solution*; the solution is blue and clear. Heat to boiling; a copious red precipitate is formed.

Tests

Appearance and odour of solution. Dissolve 10.0 g in 15 ml of *water*. The solution is clear (2.4.1), not more intensely coloured than reference solution BYS7 (2.4.1), and is odourless.

Acidity or alkalinity. Dissolve 6.0 g in 25 ml of *carbon dioxide-free water* and add 0.3 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Specific optical rotation (2.4.22). + 52.5° to + 53.3°, determined in a solution prepared by dissolving 10.0 g in 80 ml of *water*,

adding 0.2 ml of *5 M ammonia*, mixing well, allowing to stand for 30 minutes and diluting to 100.0 ml with *water*.

Arsenic (2.3.10). Dissolve 10.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). A solution prepared by dissolving 4.0 g in 10 ml of *water*, 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml, complies with the limit test for heavy metals, Method A (5 ppm).

Chlorides (2.3.12). 20 ml of a 10.0 per cent w/v solution (solution A) complies with the limit test for chlorides (125 ppm).

Sulphates (2.3.17). 7.5 ml of solution A diluted to 15 ml with *distill water* complies with the limit test for sulphates (200 ppm).

Sulphite. Dissolve 5.0 g in 40 ml of *water*, add 2.0 ml of *0.1 M sodium hydroxide* and dilute to 50.0 ml with *water*. To 10.0 ml of the solution add 1 ml of a 31 per cent w/v solution of *hydrochloric acid*, 2.0 ml of *decolourised magenta reagent* and 2.0 ml of a 0.5 per cent v/v solution of *formaldehyde solution*. Allow to stand for 30 minutes and measure the absorbance of the resulting solution at the maximum at about 583 nm (2.4.7). The absorbance is not more than that of a standard prepared in the following manner. Dissolve 76 mg of *sodium metabisulphite* in sufficient *water* to produce 50.0 ml, dilute 5.0 ml of the solution to 100.0 ml and to 3.0 ml of resulting solution add 4.0 ml of *0.1 M sodium hydroxide* and dilute to 100.0 ml with *water*. Immediately treat 10.0 ml of the resulting solution in the same manner as the test solution beginning at the words "add 1 ml of a 31 per cent w/v solution.....". Use as the blank for both measurements a solution prepared in the same manner using 10 ml of *water*.

Barium. To 10 ml of solution A add 1 ml of *1 M sulphuric acid*. Examine exactly after 1 hour; any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution A and 1 ml of *water*.

Foreign sugars, soluble starch and dextrins. Boil 1.0 g in 30 ml of *ethanol (90 per cent)* to dissolve. The appearance of the solution does not change on cooling.

Sulphated ash (2.3.18). Not more than 0.1 per cent determined by the following method. Dissolve 5.0 g in 5 ml of *water*, add 2 ml of *sulphuric acid*, evaporate to dryness and ignite to constant weight. If necessary, repeat the heating with the *sulphuric acid*.

Water (2.3.43). Not more than 1.0 per cent (anhydrous form) and 7.0 to 9.5 per cent (monohydrate), determined on 0.5 g.

Dextrose intended for use in the manufacture of parenteral preparations complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Units per ml of a 5 per cent w/v solution in *water for injections*.

Storage. Store protected from moisture.

Labelling. The label states (1) whether the material is monohydrate or is in the anhydrous form; (2) whether or not the contents are intended for use in the manufacture of parenteral preparations.

Dextrose Injection

Dextrose Intravenous Infusion; Glucose Intravenous Infusion

Dextrose Injection is a sterile solution of Dextrose in Water for Injections.

Dextrose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous dextrose, $C_6H_{12}O_6$.

Usual strengths. 5, 10, 25, and 50 per cent w/v.

Description. A clear, colourless solution. Solutions containing 20.0 per cent w/v or more of Dextrose may be not more than faintly straw-coloured.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution is blue and clear. Heat to boiling; a copious red precipitate is formed.

B. The solution prepared as directed in the Assay is dextrorotatory.

Tests

pH (2.4.24). 3.5 to 6.5, determined in a solution diluted, if necessary, with *water for injections* to contain not more than the equivalent of 5 per cent w/v of Dextrose and to which 0.30 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

5-Hydroxymethylfurfural and related substances. Dilute a volume containing 1.0 g of Dextrose to 250.0 ml with *water*. Absorbance of the resulting solution at the maximum at about 284 nm (2.4.7), not more than 0.25.

Heavy metals (2.3.13). A solution prepared by evaporating a volume containing 4 g of Dextrose to 10 ml and adding 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (5 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Units per ml for preparations containing 5 per cent w/v or less of

Dextrose. Dilute injections containing more than 5 per cent w/v of Dextrose with sufficient *water BET* so as to contain 5 per cent w/v of Dextose.

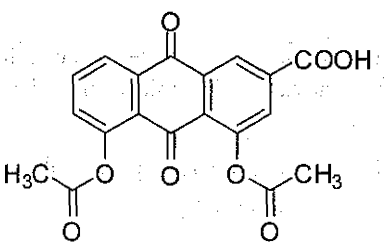
Other tests. Comply with the tests stated under Parenteral Preparations (Infusions).

Assay. To a measured volume containing between 2 g and 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$, in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the strength as the percentage w/v of anhydrous dextrose, $C_6H_{12}O_6$; (2) that the injection should not be used if it contains visible solid particles.

Diacerein



$C_{19}H_{12}O_8$ Mol. Wt. 368.3

Diacerein is 9,10-dihydro-4,5-dihydroxy-9,10-dioxo-2-anthranoic acid diacetate.

Diacerein contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{12}O_8$, calculated on the anhydrous basis.

Category. Antirheumatic.

Description. A fine yellow powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diacerein IPRS* or with the reference spectrum of diacerein.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel *GF254*.

Mobile phase. A mixture of 60 volumes of *2-propanol*, 30 volumes of *ethyl acetate* and 2 volumes of *water*.

Test solution. Dissolve about 10 mg of the substance under examination in 100.0 ml of *acetone*.

Reference solution. A 0.01 per cent w/v solution of *diacerein IPRS* in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 5.5, determined in a 1.0 per cent w/v suspension.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water* and 40 volumes of *acetonitrile*.

Test solution. Dissolve about 25 mg of the substance under examination in 10 ml of *N,N-dimethylacetamide* and dilute to 25.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *triacetyl aloe-emodin IPRS* in 5 ml of *N,N-dimethylacetamide* and dilute to 25 ml with the solvent mixture. Dilute 5.0 ml of the solution to 200.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Thermo BDS C18),
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of 0.1 per cent v/v solution of *triethylamine* in *water*, adjusted to pH 2.5 with *trifluroacetic acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of the

peak due to triacetyl aloe-emodin is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with reference solution (a) (1.5 per cent).

Aloe-emodin content. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 100 mg of the substance under examination into a 250-ml conical flask. Add 25.3 ml of *0.5 M sodium hydroxide.* Stir well for atleast 10 minutes and add 74.7 ml of *glycine solution.* Adjusted to pH 9.5 with *0.5 M sodium hydroxide.* Transfer this solution in 150 ml separating funnel. Extract the solution three times with 8.5 ml of *chloroform.* Collect the extracts and wash with 10 ml of buffer pH 9.5 and then with 10 ml of *0.01 M sulphuric acid.* Filter well through *anhydrous sodium sulphate.* Evaporate at 40° to dryness on water-bath. Dissolve the residue in 1 ml of *methanol* and 1 ml of the mobile phase, filter.

Reference solution. Dissolve 100 mg of *aloe-emodin IPRS* in 20 ml of *N, N-Dimethylacetamide* and dilute to 100 ml with *methanol.* Dilute 5 ml of the solution to 100 ml with *methanol.* Further dilute 5.0 ml of the solution to 50 ml with *methanol.* Transfer 1.0 ml of the solution into a 250-ml conical flask and add 25.3 ml of *0.5 M sodium hydroxide.* Mix well for atleast 10 minutes and add 74.7 ml of *glycine solution.* Adjusted to pH 9.5 with *0.5 M sodium hydroxide* and transfer this solution to 150-ml separating funnel. Extract the solution three times with 8.5 ml of *chloroform.* Collect the extracts and wash with 10 ml of buffer pH 9.5 and then with 10 ml of *0.005 M sulphuric acid.* Filter well through *anhydrous sodium sulphate.* Evaporate at 40° to dryness on water-bath. Dissolve the residue in 1 ml of *methanol* and 1 ml of the mobile phase, filter.

Use chromatographic system as described under Related substances using injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 20.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of peak corresponding to aloe-emodin is not more than the area of the principal peak in the chromatogram obtained with the reference solution (50 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals Method B, (10 ppm).

Chromium. Not more than 5 ppm.

Determine by atomic absorption spectrophotometry (2.4.2), Method A, using a solution prepared by dissolving 0.5 g of the substance under examination in *1 M nitric acid.* Measure the absorbance at 357.9 nm using chromium hollow cathode lamp and an air-acetylene flame. For the standard solutions dissolve 2.82 g of dried *potassium dichromate* at 120° for 4 hours in 1000 ml of *water* and dilute suitably with *1 M nitric acid* for standard solutions.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water* and 40 volumes of *acetonitrile.*

Test solution. Dissolve about 50 mg of the substance under examination in 100 ml of *N, N-dimethylacetamide.* Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. Dissolve 50 mg of *diacerein IPRS* in 100 ml of *N, N-dimethylacetamide.* Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{12}O_8$.

Storage. Store protected from light.

Diacerein Capsules

Diacerein Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diacerein, $C_{19}H_{12}O_8$.

Usual strength. 50 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of citrate buffer pH 6.0, prepared by dissolving 1.816 g of *citric acid* and 11.91 g of *trisodium citrate* in

1000 ml of *water*, adjusted to pH 6.0 with *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid*,

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary with dissolution medium at 340 nm (2.4.7). Calculate the content of diacerein, $C_{19}H_{12}O_8$ in the medium from the absorbance obtained from a solution prepared by dissolving 25 mg of *diacerein IPRS* in 10.0 ml of *acetonitrile* and dilute to 100 ml with dissolution medium. Dilute 5.0 ml of the solution to 50 ml with the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{19}H_{12}O_8$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Store and inject the solutions at 4°.

Solvent mixture (a). 60 volumes of *acetonitrile* and 20 volumes of *water*.

Solvent mixture (b). 20 volumes of *tetrahydrofuran*, 60 volumes of *acetonitrile* and 20 volumes of *water*.

Test solution. Disperse the contents of capsules containing about 25 mg of Diacerein in 10 ml of *tetrahydrofuran*, sonicate and dilute to 50.0 ml with solvent mixture (a), filter.

Reference solution (a). Dissolve about 25 mg of the *diacerein IPRS* in 10.0 ml of *tetrahydrofuran*, sonicate and dilute to 50.0 ml with solvent mixture (a).

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with solvent mixture (b). Dilute 5.0 ml of the solution to 50.0 ml with the same solvent.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as Waters X-terra RP18),
- mobile phase: a mixture of 62 volumes of a buffer solution prepared by diluting 2 ml of *orthophosphoric acid* in 1000 ml of *water* and 38 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 2.0 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of buffer pH 3.0 prepared by diluting 1.4 ml of *triethylamine* in 100 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 50 volumes of *acetonitrile*.

Test solution. Disperse the contents of capsules containing about 50 mg of Diacerein in 200 ml of solvent mixture, sonicate for 10 minutes and dilute to 250.0 ml with the solvent mixture, filter. Dilute 5.0 ml of the solution to 20.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *diacerein IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3),
- mobile phase: a mixture of 75 volumes of buffer solution prepared by diluting 1.4 ml of *triethylamine* in 100 ml of *water*, adjusted to pH 7.0 with *orthophosphoric acid* and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

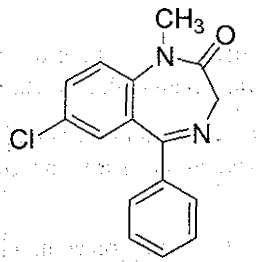
Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{12}O_8$ in the capsules.

Storage. Store protected from moisture.

Diazepam



$C_{16}H_{13}ClN_2O$ Mol. Wt. 284.7

Diazepam is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4- benzodiazepin-2-one.

Diazepam contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{16}H_{13}ClN_2O$, calculated on the dried basis.

Category. Anxiolytic; sedative; anticonvulsant.

Description. A white or almost white to pale yellow, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diazepam IPRS* or with the reference spectrum of diazepam.

B. *Measure the absorbances in subdued light immediately after preparation of the solution.*

When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.05 M methanolic sulphuric acid* shows absorption maxima at 241 nm and 284 nm; absorbance at 241 nm, about 0.5 and at 284 nm, about 0.23.

C. *Measure the absorbance in subdued light immediately after preparation of the solution.*

When examined in the range 325 nm to 400 nm (2.4.7), a 0.0025 per cent w/v solution in *0.05 M methanolic sulphuric acid* shows an absorption maximum only at 366 nm, between 0.35 and 0.39.

D. Determine by the oxygen-flask method (2.3.34), using 20 mg of the substance under examination and 5 ml of *dilute sodium hydroxide solution* as the absorbing liquid. When the process is complete, acidify the solution with *dilute sulphuric acid* and boil gently for 2 minutes; the solution gives the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 10 mg of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol.*

Reference solution (a). A solution containing 0.01 per cent w/v each of *nordazepam IPRS* and *diazepam IPRS* in *methanol.*

Reference solution (b). A solution containing 0.0001 per cent w/v of *diazepam impurity B IPRS (3-amino-6-chloro-1-methyl-4-phenylcarbostyril IPRS)*, 0.00001 per cent w/v of *diazepam impurity A IPRS (2-methylamino-5-chlorobenzophenone IPRS)* and 0.0003 per cent w/v of *nordazepam IPRS* in *methanol.*

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile*, 40 volumes of *water* and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 255 nm,
- injection volume: 10 µl.

The relative retention time with reference to diazepam for nordazepam is about 0.76.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to nordazepam and diazepam is not less than 4.0, the column efficiency is not less than 5000 theoretical plates for the principal peak and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to diazepam impurity A is not more than 0.01 per cent, the area of peak corresponding to diazepam impurity B is not more than 0.1 per cent and the area of peak corresponding to nordazepam is not more than 0.3 per cent, the area of any other secondary peak is not more than 0.1 per cent and the sum of the areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure of 1.5 to 2.5 kPa for 4 hours.

Assay. Dissolve 0.25 g in 80 ml of *anhydrous glacial acetic acid* with the aid of heat, if necessary and cool. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02847 g of $C_{16}H_{13}ClN_2O$.

Storage. Store protected from light.

Diazepam Capsules

Diazepam Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diazepam, $C_{16}H_{13}ClN_2O$.

Usual strengths. 2 mg; 5 mg; 10 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 100 volumes of *chloroform* and 10 volumes of *methanol.*

Test solution. Shake a quantity of the contents of the capsules with sufficient *methanol* to produce a solution containing 0.5 per cent w/v of Diazepam, allow to settle and decant the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of *diazepam IPRS* in *methanol.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 10 per cent v/v solution of *sulphuric acid* in *ethanol*, heat at 105° for 10 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows two absorption maxima at 242 nm 284 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid.* Measure the absorbance of the resulting solution at the maximum at about 242 nm (2.4.7). Calculate the content of diazepam, $C_{16}H_{13}ClN_2O$ in the medium from the absorbance obtained from a solution of known concentration of *diazepam IPRS.*

Q. Not less than 85 per cent of the stated amount of $C_{16}H_{13}ClN_2O$.

Related substances and decomposition products. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of equal volumes of *hexane* and *ethyl acetate.*

NOTE— Prepare the following solutions freshly.

Test solution. Shake a quantity of the contents of the capsules containing 50 mg of Diazepam with 5 ml of *acetone* and filter.

Reference solution. Dilute 1 volume of the test solution to 50 volumes with *acetone.*

Apply to the plate 20 µl of the test solution and 5 µl of the reference solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Uniformity of content. Complies with the test stated under Capsules using the following method of analysis. Weigh an intact capsule. Open the capsule without losing any part of the shell and transfer the contents as completely as possible to a 100-ml volumetric flask. Weigh the shell, remove any retained contents and reweigh the shell. To the flask add 1 ml of *water*, mix and allow to stand for 15 minutes. Add 80 ml of a 0.5 per cent w/v solution of *sulphuric acid* in *methanol*, shake for 15 minutes, add sufficient of the *methanolic sulphuric acid* to produce 100.0 ml and filter. Dilute suitably, if necessary and measure the absorbance at the maximum at 284 nm (2.4.7). Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the specific absorbance at 284 nm, making an appropriate adjustment for any retained capsule content.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 10 mg of Diazepam, add 5 ml of *water* and complete the test as described under Uniformity of content beginning at the words "mix and allow to stand for 15 minutes....". Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the specific absorbance at 284 nm.

Storage. Store protected from light.

Diazepam Injection

Diazepam Injection is a sterile solution of Diazepam in Water for Injections or other suitable solvent.

Diazepam Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diazepam, $C_{16}H_{13}ClN_2O$.

Usual strengths. 5 mg per ml; 10 mg per ml.

Description. A clear, colourless or almost colourless solution.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 100 volumes of *chloroform* and 10 volumes of *methanol.*

Test solution. Dilute if necessary, a measured volume of the injection with *water* to produce a solution containing 5 mg of Diazepam per ml.

Reference solution. A 0.5 per cent w/v solution of *diazepam IPRS* in *methanol.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 10 per cent v/v solution of *sulphuric acid* in *ethanol*, heat at 105° for 10 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 242 and at about 284 nm.

Tests

pH (2.4.24). 6.2 to 6.9.

Bacterial endotoxins (2.2.3). Not more than 11.6 Endotoxin Units per mg of diazepam.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume containing 10 mg of Diazepam, add 20 ml of *buffer solution pH 7.0* and extract with four quantities, each of 20 ml, of *chloroform*, passing each extract through about 5 g of *anhydrous sodium sulphate* and dilute the combined chloroform extracts to 100.0 ml with *chloroform*. Evaporate 10.0 ml of the solution to dryness under nitrogen, add sufficient volume of a 0.5 per cent w/v solution of *sulphuric acid* in *methanol* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 284 nm (2.4.7). Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the specific absorbance at 284 nm.

Storage. Store in single dose or multiple dose containers protected from light.

Diazepam Tablets

Diazepam Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diazepam, $C_{16}H_{13}ClN_2O$.

Usual strengths. 2 mg; 5 mg; 10 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Shake a quantity of the powdered tablets with sufficient *methanol* to produce a solution containing 0.5 per cent w/v of Diazepam, allow to settle and decant the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of *diazepam IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 10 per cent v/v solution of *sulphuric acid* in *ethanol*, heat at 105° for 10 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 230 to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 242 and at about 284 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 242 nm (2.4.7). Calculate the content of diazepam, $C_{16}H_{13}ClN_2O$ in the medium from the absorbance obtained from a solution of known concentration of *diazepam IPRS*.

Q. Not less than 85 per cent of the stated amount of $C_{16}H_{13}ClN_2O$.

Related substances and decomposition products. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of equal volumes of *hexane* and *ethyl acetate*.

Test solution. Prepare freshly by shaking a quantity of the powdered tablets containing 50 mg of Diazepam with 5 ml of *ethanol (95 per cent)* and filtering.

Reference solution. Dilute 1.0 ml of the test solution to 50.0 ml with *ethanol (95 per cent)*.

Apply to the plate 20 µl of the test solution and 5 µl of the reference solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Uniformity of content. Complies with the test stated under Tablets.

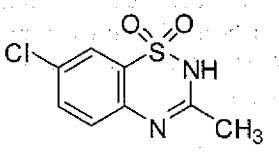
Powder one tablet, add 1 ml of *water*, mix and allow to stand for 15 minutes. Add 80 ml of a 0.5 per cent w/v solution of *sulphuric acid* in *methanol*, shake for 15 minutes, add sufficient of the *methanolic sulphuric acid* to produce 100.0 ml and filter. Dilute suitably, if necessary and measure the absorbance at the maximum at 284 nm (2.4.7). Calculate the content of $C_{16}H_{13}ClN_2O$ in the tablet taking 450 as the specific absorbance at 284 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 10 mg of Diazepam, add 5 ml of *water* and complete the test as described under Uniformity of content beginning at the words "mix and allow to stand for 15 minutes....". Calculate the content of $C_{16}H_{13}ClN_2O$ taking 450 as the specific absorbance at 284 nm.

Storage. Store protected from light.

Diazoxide



$C_8H_7ClN_2O_2S$ Mol. Wt. 230.7

Diazoxide is 7-chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide.

Diazoxide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_8H_7ClN_2O_2S$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or almost white, fine or crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diazoxide IPRS* or with the reference spectrum of diazoxide.

B. Dissolve 0.05 g in 5 ml of *1 M sodium hydroxide*, dilute to 50 ml with *water*. Dilute 1.0 ml of the solution to 100 ml with *0.1 M sodium hydroxide*. When examined in the range 230 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum at 280 nm and a shoulder at 304 nm. The specific absorbance is 570 to 610.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to the principal spot in the chromatogram obtained with reference solution (b).

D. Dissolve about 20 mg in a mixture of 5 ml of *hydrochloric acid* and 10 ml of *water*. Add 0.1 g of *zinc powder*. Boil for 5 minutes, cool and filter. To the filtrate add 2 ml of a 0.1 per cent w/v solution of *sodium nitrite* and mix. Allow to stand for 1 minute and add 1 ml of a 0.5 per cent solution of *naphthylethylenediamine dihydrochloride*. A red or violet-red colour develops.

Tests

Appearance of solution. Dissolve 0.4 g in 2 ml of *1 M sodium hydroxide* and dilute to 20 ml with *water*. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

Acidity or alkalinity. To 0.5 g of the substance under examination, add 30 ml of *carbon dioxide-free water*, shake for 2 minutes and filter. To 10 ml of the filtrate add 0.2 ml of *0.01 M sodium hydroxide* and 0.15 ml of *methyl red solution*, the solution is yellow. Not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red.

Related substance. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 7 volumes of *ammonia*, 25 volumes of *methanol* and 68 volumes of *chloroform*.

Test solution (a). Dissolve 0.1 g of the substance under examination in a mixture of 0.5 ml of *1 M sodium hydroxide* and 1 ml of *methanol* and dilute to 5 ml with *methanol*.

Test solution (b). Dilute 1 ml of test solution (a) to 5 ml with a mixture of 1 volume of *1 M sodium hydroxide* and 9 volumes of *methanol*.

Reference solution (a). Dilute 0.5 ml of test solution (a) to 100 ml with a mixture of 1 volume of *1 M sodium hydroxide* and 9 volumes of *methanol*.

Reference solution (b). Dissolve 20 mg of *diazoxide IPRS* in a mixture of 0.5 ml of *1M sodium hydroxide* and 1 ml of *methanol* and dilute to 5 ml with *methanol* .

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.2 g with gentle heating in 50 ml of a mixture of 1 volume of *water* and 2 volumes of *dimethylformamide*.

Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02307 g of $C_8H_7ClN_2O_2S$.

Diazoxide Tablets

Diazoxide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diazoxide, $C_8H_7ClN_2O_2S$.

Usual strength. 50 mg.

Identification

Shake a quantity of the powdered tablets containing 0.2 g of Diazoxide with 50 ml of *absolute ethanol*, filter and evaporate the filtrate to dryness at a pressure of 2 kPa. The residue complies with the following tests.

A. When examined in the range 230 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorbance maximum only at 280 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *toluene*, 30 volumes of *ether* and 20 volumes of *acetone*.

Test solution. A 0.02 per cent w/v solution of the residue in *methanol*.

Reference solution. A 0.02 per cent w/v solution of *diazoxide IPRS* in *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the solvent has evaporated, examine under ultraviolet light at 254 nm and then spray the dried plate with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed glass tank for 15 minutes (the nitrous fumes may be generated by adding *7 M sulphuric acid* dropwise to a solution containing 10 per cent w/v of *sodium nitrite* and 3 per cent w/v of *potassium iodide*. Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)*. If necessary allow to dry and repeat the spraying. The principal spot in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 7 volumes of *18 M ammonia*, 25 volumes of *methanol* and 68 volumes of *chloroform*.

Test solution. Shake a quantity of the powdered tablets containing 0.75 g of Diazoxide with 40 ml of *0.1 M sodium hydroxide* for 30 minutes, filter and dilute the filtrate to 50 ml with *0.1 M sodium hydroxide*.

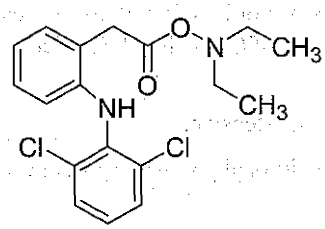
Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with *0.1 M sodium hydroxide*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.05 g of Diazoxide, add 70 ml of *methanol*, shake for 1 hour, add sufficient *methanol* to produce 100 ml, mix and filter. Dilute 5 ml of the filtrate to 250 ml with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at 280 nm (2.4.7). Calculate the content of $C_8H_7ClN_2O_2S$ taking 585 as the specific absorbance at 280 nm.

Diclofenac Diethylamine



$C_{18}H_{22}Cl_2N_2O_2$ Mol. Wt. 369.3

Diclofenac Diethylamine is diethylammonium 2-[(2,6-dichloroanilino)phenyl]acetate.

Diclofenac Diethylamine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{22}Cl_2N_2O_2$, calculated on the dried basis.

Category. Analgesic; antiinflammatory.

Description. A white to light beige, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diclofenac diethylamine IPRS* or with the reference spectrum of diclofenac diethylamine.

B. Determine by thin-layer chromatography (2.4.17), using the plate coated with *silica gel GF254*.

Mobile phase. A mixture of 0.1 volume of *hydrochloric acid*, 1 volume of *water*, 6 volumes of *glacial acetic acid* and 11 volumes of *ethyl acetate*.

Test solution. Dissolve 1.0 g of the substance under examination in 100.0 ml of *methanol*.

Reference solution. A 1.0 per cent w/v solution of *diclofenac diethylamine IPRS* in a *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. After development, dry the plate at 105°, spray with *ninhydrin solution* and heat at 110° for 15 minutes, the chromatogram obtained with the reference solution shows two clearly separated spots.The two principal spots in the chromatogram obtained with the test solution correspond to that in the chromatogram obtained with the reference solution.

C. Melting point (2.4.21). About 154° with decomposition.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *methanol* is clear (2.4.1), The absorption of the solution measured at 440 nm (2.4.7) is not more than 0.05.

pH (2.4.24). 6.4 to 8.4, determined in a 1.0 per cent w/v solution in *ethanol* (10 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the same solvent.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v of *diclofenac impurity A (1(2,6 dichlorophenyl)-1,3-dihydro-2H-indol-2-one) IPRS* and add 1.0 ml of test solution in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octylsilane silica gel (5 µm),
- mobile phase: 34 volumes of a mixture of equal volumes of a 0.1 per cent w/v solution of *orthophosphoric acid* and a 0.16 per cent w/v solution of *sodium dihydrogen orthophosphate dihydrate*, adjusted to pH 2.5 and 66 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The retention times are about 25 minutes for diclofenac and about 12 minutes for diclofenac impurity A.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to diclofenac and diclofenac impurity A is not less than 6.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent); the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at a pressure not exceeding 1 kPa for 24 hours.

Assay. Dissolve 0.5 g in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03693 g of $C_{18}H_{22}Cl_2N_2O_2$.

Storage. Store protected from light and moisture.

Diclofenac Gel

Diclofenac Gel is a Diclofenac Diethylamine in a suitable base.

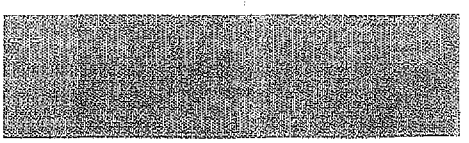
Diclofenac Gel contains not less than 90.0 per cent and not more than 110.0 per cent of the stated equivalent amount of diclofenac diethylamine, $C_{18}H_{22}Cl_2N_2O_2$.

Usual strength. 1.16 per cent w/w.

Identification

Determine by thin-layer chromatography (2.4.17), using the plate coated with *silica gel GF254*.

Mobile phase. A mixture of 0.1 volume of *hydrochloric acid*, 1 volume of *water*, 6 volumes of *glacial acetic acid* and 11 volumes of *ethyl acetate*.



Test solution. Disperse a quantity of the gel containing 50 mg of Diclofenac Diethylamine in 12.5 ml of *0.5M sodium chloride* and shake until a homogeneous suspension is obtained. Add 12.5 ml of *chloroform*, shake well and mix with the aid of ultrasound for 5 minutes. Allow the layers to separate, filter the chloroform layer through a suitable glass fibre filter and use the filtrate.

Reference solution. A 0.2 per cent w/v solution of *diclofenac diethylamine IPRS* in *chloroform*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. After development, dry the plate at 105°, spray with *ninhydrin solution* and heat at 110° for 15 minutes. The two principal spots in the chromatogram obtained with the test solution correspond to that in the chromatogram obtained with the reference solution

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the gel containing 50 mg of Diclofenac Diethylamine with 50 ml of *acetone* for 10 minutes, filter and evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 10.0 ml of a mixture of 40 volumes of *water* and 60 volumes of *methanol* , dilute 1.0 ml of the solution to 5.0 ml with the mobile phase and filter through a suitable glass fibre filter.

Reference solution (a). Dilute 1.0 ml of test solution to 100.0 ml with *methanol.*

Reference solution (b). A 0.01 per cent w/v solution each of *diclofenac sodium IPRS* and *diclofenac impurity A IPRS [1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one IPRS]* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octylsilane silica gel (5 µm),
- mobile phase: 34 volumes of a mixture of equal volumes of a 0.1 per cent w/v solution of *orthophosphoric acid* and a 0.16 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5 and 66 volumes of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The retention times are about 25 minutes for diclofenac and about 12 minutes for diclofenac impurity A.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to diclofenac and diclofenac impurity A is not less than 6.5.

Inject reference solution (a) and the test solution. Run the chromatogram 1.5 times the retention time of principal peak. In the chromatogram obtained with test solution, the area of any secondary peak is not more than 0.5 times the areas of principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of all the secondary peaks is not more than the area of principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Gels.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the gel containing 50 mg of Diclofenac Diethylamine with 50 ml of *acetone* for 10 minutes, filter and evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 100.0 ml of a mixture of 40 volumes of *water* and 60 volumes of *methanol.* Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase and filter through a glass fibre filter (Whatman GF/C is suitable).

Reference solution (a). A 0.005 per cent w/v solution of *diclofenac sodium IPRS* in *methanol.*

Reference solution (b). A 0.01 per cent w/v solution each of *diclofenac sodium IPRS* and *diclofenac impurity A IPRS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octylsilane silica gel (5 µm),
- mobile phase: 20 volumes of a mixture of equal volumes of a 0.1 per cent w/v solution of *orthophosphoric acid* and a 0.16 per cent w/v solution of *sodium dihydrogen orthophosphate dihydrate*, adjusted to pH 2.5 and 80 volumes of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The retention times are about 5 minutes for diclofenac and about 4 minutes for diclofenac impurity A.

Inject reference solution (b). The test is not valid unless the resolution between the diclofenac peak and diclofenac impurity A peak is not less than 2.0.

Inject reference solution (a) and the test solution.

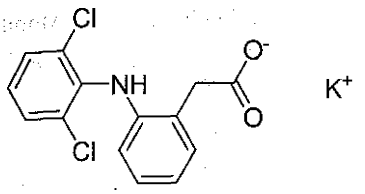
Calculate the content of $C_{18}H_{22}Cl_2N_2O_2$ in the gel.

1 mg of $C_{14}H_{10}Cl_2NNaO_2$ is equivalent to 1.1609 mg of $C_{18}H_{22}Cl_2N_2O_2$.

Storage. Store in a cool, dry place, at a temperature not exceeding 30°.

Labelling. The label states the quantity in terms of diclofenac diethylamine as well as the equivalent amount of diclofenac sodium.

Diclofenac Potassium



$C_{14}H_{10}Cl_2KNO_2$ Mol. Wt. 334.2

Diclofenac Potassium is benzene acetic acid, 2-[(2,6-dichlorophenyl)amino]-,monopotassium salt.

Diclofenac Potassium contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{10}Cl_2KNO_2$, calculated on the dried basis.

Category. Analgesic, anti-inflammatory.

Description. A white to off white or slightly yellowish crystalline powder, slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diclofenac potassium IPRS* or with the reference spectrum of diclofenac potassium.

B. When examined in the range 230 nm to 360 nm (2.4.7). A 0.001 per cent w/v solution in *methanol* shows an absorption maximum as obtained with *diclofenac potassium IPRS* of the same concentration.

C. To 0.5 g, add 10 ml of *water*, stir and add water until the substance is dissolved. Add 2 ml of *7M hydrochloride acid*, stir for 60 minutes, and filter with the aid of a vacuum. Neutralize with *5M sodium hydroxide*. Take 1 ml of the solution, add 1 ml of *2 M acetic acid* and 1ml of freshly prepared 10 per cent w/v solution of *sodium cobaltinitrite*. A yellow or orange yellow precipitate is formed immediately.

Tests

pH (2.4.24). 7.0 to 8.5, determined in 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14)

Buffer solution. A mixture of equal volumes of *0.01M orthophosphoric acid* and *0.01M sodium dihydrogen orthophosphate*, adjusted to pH 2.5, with additional portions of the appropriate components.

Solvent mixture. 70 volumes of *methanol* and 30 volumes of *water*.

Test solution. Dissolve 75 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.004 per cent w/v of *diehtyl phthalate*, 0.05 per cent w/v of *diclofenac potassium IPRS* and 0.00225 per cent w/v of *diclofenac related compound A IPRS {N-(2,6-dichlorophenyl)indolin-2-one}* in the solvent mixture.

Reference solution (b). A 0.015 per cent w/v solution of *diclofenac related compound A IPRS* in *methanol*. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of the buffer solution,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 30 µl.

The relative retention time with reference to diclofenac potassium for diethyl phthalate and diclofenac related compound A is about 0.5 and 0.7 respectively.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to diethyl phthalate and diclofenac related compound A is not less than 4.0 in the chromatogram obtained with reference solution (a). The relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to diclofenac related compound A is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 105° under vacuum for 3 hours.

Assay. Dissolve 0.3 g in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1M perchloric acid*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03342 g of $C_{14}H_{10}Cl_2KNO_2$.

Storage. Store protected from light and moisture, at temperature not exceeding 30°.

Diclofenac Potassium Tablets

Diclofenac Potassium Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diclofenac potassium, $C_{14}H_{10}Cl_2KNO_2$.

Usual strengths. 25 mg; 50 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *intestinal fluid, simulated* (without enzyme),
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with medium, if necessary, at the maximum at about 276 nm (2.4.7). Calculate the content of $C_{14}H_{10}Cl_2KNO_2$ in the medium from the absorbance obtained from a solution of known concentration of *diclofenac potassium IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{14}H_{10}Cl_2KNO_2$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions, protected from light.

Solvent mixture. Equal volumes of *acetonitrile* and *water.*

Test solution. Disperse a quantity of powdered tablets containing 100 mg of Diclofenac Potassium in 80 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A solution containing 0.0001 per cent w/v, each of, *diclofenac potassium IPRS* and *diclofenac related compound A IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm x 2.0 mm, packed with octadecylsilane bonded to porous silica (1.9 µm).
- column temperature: 35°,
- mobile phase: A. *0.01 M ammonium acetate*, adjusted to pH 5.3 with *glacial acetic acid*,
  B. *acetonitrile*,
- flow rate: 0.3 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 1 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
0.5	70	30
8.5	5	95
10	5	95
10.1	70	30
15	70	30

Name	Relative retention time
Oxindole[1]*	0.4
Diclofenac	1.0
Diclofenac related compound D[2]*	1.04
Diclofenac related compound A[3]	1.48
Diclofenac alcohol analog[4]*	1.55
Diclofenac benzaldehyde analog[5]*	1.81

*Process-related impurities, included for identification only, not to be included in total impurities.

[1]1,3-Dihydro-2*H*-indol-2-one,

[2]2-{2[(2-Bromo-6-chlorophenyl)amino]phenyl}acetic acid,

[3]*N*-(2,6-Dichlorophenyl)indolin-2-one (diclofenac lactum),

[4]{2-[(2,6-Dichlorophenyl)amino]phenyl}methanol,

[5]2-[(2,6-Dichlorophenyl)amino]benzaldehyde.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent and the signal-to-noise ratio for the principal peak is not less than 10.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to diclofenac related compound A is not more than 5 times the area of diclofenac related compound A peak in the chromatogram obtained with the reference solution (0.5 per cent), the area of any other secondary peak is not more than 5 times of the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 15 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Diclofenac Potassium in 80 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.02 per cent w/v solution of *diclofenac potassium IPRS* in the solvent mixture.

– spectrophotometer set at 280 nm,

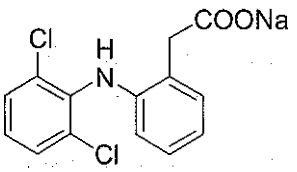
Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.2 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{10}Cl_2KNO_2$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Diclofenac Sodium



$C_{14}H_{10}Cl_2NNaO_2$ Mol. Wt. 318.1

Diclofenac Sodium is sodium 2-[(2,6-dichlorophenyl)-amino]phenylacetate.

Diclofenac Sodium contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{14}H_{10}Cl_2NNaO_2$, calculated on the dried basis.

Category. Analgesic; anti-inflammatory.

Description. A white to slightly yellowish crystalline powder; slightly hygroscopic.

Identification

Test A may be omitted if tests B, C, and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diclofenac sodium IPRS* or with the reference spectrum of diclofenac sodium.

B. To 1 ml of a 0.4 per cent w/v solution in *methanol* add 1 ml of *nitric acid;* a dark red colour develops.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution(a).

D. A 1.0 per cent w/v solution gives the reaction of sodium salts (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 6.5 to 8.5, determined on a 1.0 per cent w/v solution.

Light absorption (2.4.7). Absorbance of a 5.0 per cent w/v solution in *methanol* at 440 nm, not more than 0.050.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *methanol* and dilute to 50 ml with the same solvent.

Reference solution (a). A 0.0002 per cent w/v solution of *diclofenac sodium IPRS* in *methanol.*

Reference solution (b). A solution containing 0.0005 per cent w/v each of *diclofenac sodium IPRS* and *1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one IPRS (diclofenac impurity A IPRS)* in the mobile phase.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm, packed with end-capped octylsilane bonded to porous silica (5 µm),
– mobile phase: a mixture of 34 volumes of a solution containing 0.5 g per litre of *phosphoric acid* and 0.8 g per litre of *sodium dihydrogen phosphate* adjusted to pH 2.5 with *phosphoric acid*, and 66 volumes of *methanol*,
– flow rate: 1 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to diclofenac and diclofenac impurity A is not less than 6.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution: the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent); the sum of the areas of all the secondary peaks is not more than 2.5 times that of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (a).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.2 g in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03181 g of $C_{14}H_{10}Cl_2NNaO_2$.

Storage. Store protected from light.

Diclofenac Injection

Diclofenac Sodium Injection

Diclofenac Injection is a sterile solution of Diclofenac Sodium in Water for Injections. It may contain Propylene Glycol, Benzyl Alcohol and sufficient Sodium Hydroxide to adjust the pH of the solution.

Diclofenac Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$.

Usual strength. 25 mg per ml.

Description. A clear, colourless to yellowish liquid.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 5 volumes of *acetone* and 5 volumes of *formic acid* in a saturated chamber.

Test solution. Dilute a suitable volume of the injection containing 25 mg of Diclofenac Sodium to 10 ml with *methanol*.

Reference solution. A 0.25 per cent w/v solution of *diclofenac sodium IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. Alternatively, spray with a 0.5 per cent w/v solution of *potassium dichromate* in *sulphuric acid (20 per cent)*. By both methods of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 8.1 to 9.0.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the injection containing 50 mg of Diclofenac Sodium to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (a). A 0.005 per cent w/v solution of *diclofenac sodium IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v each of *diclofenac sodium IPRS* and *1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one IPRS (diclofenac impurity A IPRS)* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 34 volumes of a mixture of equal volumes of a 0.1 per cent w/v solution of *orthophosphoric acid* and a 0.16 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5, and 66 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between diclofenac and diclofenac impurity A is not less than 6.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ in the injection.

Diclofenac Gastro-resistant Tablets

Diclofenac Tablets; Diclofenac Sodium Gastro-resistant Tablets; Diclofenac Sodium Tablets

Diclofenac Gastro-resistant Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$. They are made gastro-resistant by enteric-coating or by other means.

Usual strengths. 25 mg; 50 mg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel 60 F254* or using a precoated *silica gel 60 F254* plate.

Mobile phase. A mixture of 100 volumes of *toluene*, 10 volumes of *hexane* and 10 volumes of *anhydrous formic acid*.

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Diclofenac Sodium with 5 ml of *methanol*, centrifuge and use the supernatant liquid.

Reference solution. A 1 per cent w/v solution of *diclofenac sodium IPRS* in *methanol*.

Apply separately to the plate 1 µl of each solution. After development, dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. Alternatively, spray the plate with a 0.5 per cent w/v solution of *potassium dichromate* in *sulphuric acid (20 per cent)*. By both methods of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing about 50 mg of Diclofenac Sodium with 70 ml of the mobile phase for 30 minutes and dilute to 100.0 ml with the mobile phase, centrifuge and filter the supernatant liquid.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 5.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v each of *diclofenac sodium IPRS* and *diclofenac impurity A (1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one) IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Zorbax C8),
- mobile phase: a mixture of 34 volumes of a solution containing a mixture of equal volumes of a 0.1 per cent w/v of *orthophosphoric acid* and a 0.16 per cent w/v of *sodium dihydrogen orthophosphate*, adjusted to pH 2.5 and 66 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The retention time of diclofenac is about 25 minutes and that of diclofenac impurity A is about 12 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to diclofenac and diclofenac impurity A is not less than 6.5.

Inject reference solution (a) and the test solution. Run the chromatogram 1.5 times the retention time of diclofenac. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent) and any peak with relative retention times of 0.67 and 0.1 with reference to the principal peak.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Diclofenac Sodium in the mobile phase, dilute to100.0 ml with the mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). A 0.005 per cent w/v solution of *diclofenac sodium IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v each of *diclofenac sodium IPRS* and *diclofenac impurity A (1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one) IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between diclofenac and diclofenac impurity A is not less than 6.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{14}H_{10}C_{l2}NNaO_2$ in the tablet.

Storage. Store protected from light.

Diclofenac Prolonged-release Tablets

Diclofenac Sodium Prolonged-release Tablets

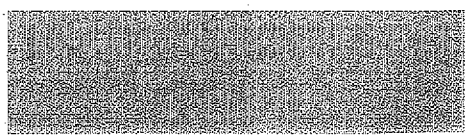
Diclofenac Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Diclofenac Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diclofenac sodium, $C_{14}H_{10}Cl_2NNaO_2$.

Usual strength. 100 mg.

Identification

Disperse a quantity of powdered tablets containing 0.15 g of Diclofenac Sodium with 0.5 ml of *glacial acetic acid* and 15 ml of *methanol* with the aid of ultrasound, filter and collect the filtrate in 15 ml of *water*. Filter the precipitate under reduced pressure, wash with four 5-ml quantities of *water* and dry the residue at 105° for 2 to 3 hours. Determine by infrared absorption spectrophotometry (2.4.6) on the residue. Compare



the spectrum with that obtained with *diclofenac sodium IPRS* treated in the same manner or with the reference spectrum of diclofenac.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Diclofenac Sodium with 70 ml of the mobile phase with the aid of ultrasound for 30 minutes and dilute to 100.0 ml with the mobile phase, centrifuge and filter.

Reference solution (a). A 0.0001 per cent w/v solution of *diclofenac sodium IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v each of *diclofenac sodium IPRS* and *diclofenac impurity A (1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one) IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 34 volumes of a solution containing a mixture of equal volumes of 0.1 per cent w/v of *orthophosphoric acid* and 0.16 per cent w/v of *sodium dihydrogen orthophosphate dihydrate*, adjusted to pH 2.5 and 66 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to diclofenac and diclofenac impurity A is not less than 6.5.

Inject reference solution (a) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.5 g of Diclofenac Sodium in *methanol* with the aid of ultrasound and dilute with the mobile phase to obtain 0.005 per cent w/v of diclofenac sodium.

Reference solution (a). A 0.005 per cent w/v solution of *diclofenac sodium IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v each of *diclofenac sodium IPRS* and *diclofenac impurity A IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of equal volumes of 0.1 per cent w/v solution of *orthophosphoric acid* and 0.16 per cent w/v solution of *sodium dihydrogen orthophosphate dihydrate*, adjusted to pH 2.5 and 80 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between diclofenac and diclofenac impurity A is not less than 2.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ in the tablet.

Storage. Store protected from light and moisture.

Diclofenac Sodium and Paracetamol Tablets

Diclofenac Sodium and Paracetamol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diclofenac sodium $C_{14}H_{10}Cl_2NNaO_2$ and paracetamol $C_8H_9NO_2$.

Usual strength. Diclofenac sodium 50 mg and Paracetamol 325 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *phosphate buffer pH 6.8*,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the mobile phase to obtain a solution having similar concentration to the reference solution.

Reference solution. A solution containing 0.0032 per cent w/v of *paracetamol IPRS* and 0.0005 per cent w/v of *diclofenac sodium IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *water*, 75 volumes of *methanol* and 1.0 volume of *glacial acetic acid*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ and $C_8H_9NO_2$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{14}H_{10}Cl_2NNaO_2$ and $C_8H_9NO_2$.

4-Aminophenol. Determine by liquid chromatography (2.4.14).

Solvent mixture A. Dissolve 4.6 g of *tetrabutylammonium hydroxide (40 per cent)* in 1000 ml of *methanol.*

Solvent mixture B. A *0.05 M disodium hydrogen orthophosphate dodecahydrate solution* .

Solvent mixture C. A *0.05 M sodium dihydrogen orthophosphate dihydrate solution.*

Test solution. Disperse a quantity of powdered tablets containing 25 mg of paracetamol in 6.3 ml of solvent mixture A, with the aid of ultrasound for 10 minutes and dilute to 25.0 ml with a mixture of equal volumes of solvent mixture B and solvent mixture C.

Reference solution. A solution containing 0.00015 per cent w/v solution, each of *4-aminophenol IPRS* and *paracetamol IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 325 volumes of solvent mixture A, 335 volumes of solvent mixture B and 340 volumes of solvent mixture C.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 20 µl.

The relative retention times are 0.75 for the peaks due to 4-aminophenol and 1.0 for paracetamol.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to 4-aminophenol is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (0.15 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 325 mg of Paracetamol to a 50-ml volumetric flask, add 30 ml of mobile phase and disperse with the aid of ultrasound for about 10 minutes, cool and dilute to 50.0 ml with the mobile phase, filter. Dilute 1.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution. A solution containing each of 0.013 per cent w/v of *paracetamol IPRS and* 0.002 per cent of *diclofenac sodium IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *water*, 75 volumes of *methanol* and 1.0 volume of *glacial acetic acid*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

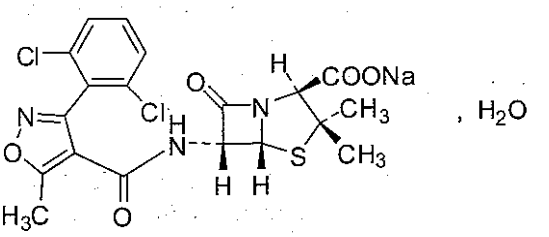
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{10}Cl_2NNaO_2$ and $C_8H_9NO_2$ in the tablets.

Storage. Store protected from light and moisture at a temperature below 30°.

Dicloxacillin Sodium



$C_{19}H_{16}Cl_2N_3NaO_5S,H_2O$ Mol. Wt. 510.3

Dicloxacillin Sodium is sodium (6*R*)-6-[3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamido] penicillanate monohydrate.

Dicloxacillin Sodium contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{19}H_{16}Cl_2N_3NaO_5S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white crystalline powder, hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicloxacillin sodium IPRS* or with the reference spectrum of dicloxacillin sodium.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 30 volumes of *acetone* and 70 volumes of a 15.4 per cent w/v solution of *ammonium acetate*, adjusted to pH 5.0 with *glacial acetic acid*.

Test solution. Dissolve 25 mg of the substance under examination in 5 ml of *water*.

Reference solution (a). A 0.5 per cent w/v solution of *dicloxacillin sodium IPRS* in *water*.

Reference solution (b). A solution containing 0.5 per cent w/v each of *cloxacillin sodium IPRS*, *dicloxacillin sodium IPRS* and *flucloxacillin sodium IPRS* in *water*.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air. Expose to iodine vapour until the spots appear and examine in daylight, the chromatogram obtained with reference solution (b) shows three clearly separated spots. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and about 15 mm in diameter. Moisten with 0.05 ml of *water* and add 2 ml of *sulphuric acid-formaldehyde reagent*. Mix the contents of the tube by swirling; the colour of the solution is slightly greenish-yellow. Place the test-tube in a water-bath for 1 minute; a yellow colour develops.

D. It gives reaction (A) of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and its absorbance at 430 nm (2.4.7) is not more than 0.04.

pH (2.4.24). 5.0 to 7.0, determined in solution A.

Specific optical rotation (2.4.22). + 128° to + 143°, determined in a 1 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Test solution (b). Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *dicloxacillin sodium IPRS* in the mobile phase.

Reference solution (b). Dilute 5.0 ml of test solution (b) to 50.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.01 per cent w/v each of *flucloxacillin sodium IPRS* and *dicloxacillin sodium IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 5.0 with *dilute sodium hydroxide solution*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to flucloxacillin and dicloxacillin is not less than 2.5.

Inject reference solution (b) and test solution (a). Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with test solution (a), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by Method A.

2-Ethylhexanoic acid. Not more than 0.8 per cent.

Determine by gas chromatography (2.4.13).

Test solution. Prepare a 0.1 per cent w/v solution of *3-cyclopropionic acid* (Internal standard) in *cyclohexane* (solution A). To 0.3 g of the substance under examination add 4.0 ml of 33 per cent v/v solution of *hydrochloric acid* and 1.0 ml of solution (A). Shake vigorously for 1 minute, centrifuge if necessary and use the clear supernatant layer.

Reference solution. Dissolve 75 mg of *2-ethylhexanoic acid* in solution A and dilute to 50 ml with solution A. To 1 ml of the solution add 4.0 ml of a 33 per cent v/v solution of *hydrochloric acid*, shake vigorously for 1 minute, centrifuge if necessary and use the clear supernatant layer.

Chromatographic system
- a wide-bore fused silica column 10 m x 0.53 mm coated with *macrogol* 20,000 *2-nitrophthalate* (film thickness 1.0 µm),
- temperature:
  column 40° from 0 to 2 minutes, 40°-200° from 2 to 7.3 minutes and 200° from 7.3 to 10.3 minutes,
  inlet port 200°and detector 300°,
- flow rate: 10 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks of *2-ethylhexanoic acid* and *3-cyclohexylpropionic acid* is not less than 2.0.

Inject 1 µl of the reference solution and the test solution.

Calculate the content of 2-ethylhexanoic acid.

Water (2.3.43). 3.0 to 4.5 per cent, determined on 0.3 g.

Dicloxacillin Sodium intended for use in the manufacture of parenteral preparations complies with the following additional tests.

Pyrogens. Complies with the test for pyrogens (2.2.8), using not less than 20 mg per kg of the rabbit's weight, dissolved in 1 ml of *water for injection.*

Assay. Determine by liquid chromatography (2.4.14), using test solution (b), reference solution (a) and chromatographic system as described under the test for Related substances.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and test solution (b).

Calculate the content of $C_{19}H_{16}Cl_2N_3NaO_5S$.

Storage. Store protected from moisture, at a temperature not exceeding 25°. If it is intended for use in manufacture of parenteral preparation, the container should be sterile, airtight and temper-proof.

Dicloxacillin Capsules

Dicloxacillin Sodium Capsules

Dicloxacillin Capsules contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of dicloxacillin, $C_{19}H_{17}Cl_2N_3O_5S$.

Usual strengths. 250 mg; 500 mg.

Identification

In the Assay, the principle peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with buffer solution and filter.

Reference solution. A 0.11 per cent w/v solution of *dicloxacillin sodium IPRS* in the buffer solution. Dilute suitably with buffer solution.

Use the chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$.

Q. Not less than 75 per cent of the stated amount of $C_{19}H_{17}Cl_2N_3O_5S$.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.1 g.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Buffer solution. Dissolve 5.44 g of *monobasic potassium phosphate* in 2000 ml of *water*, adjusted to pH 5.0 with *8 M potassium hydroxide.*

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing about 100 mg of dicloxacillin, dissolve in about 70 ml of buffer solution by stirring for 10 minutes, dilute to 100.0 ml with the buffer solution and filter.

Reference solution. A 0.11 per cent w/v solution of *dicloxacillin sodium IPRS* in the buffer solution.

Chromatographic system
- a stainless steel column 30 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of the buffer solution and 25 volumes of *acetonitrile,*
- flow rate: 2 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$ in the capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Dicloxacillin Oral Suspension

Dicloxacillin Sodium Oral Suspension

Dicloxacillin Oral Suspension is a dry mixture of Dicloxacillin with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of sealed container in the specified volume of water just before use.

Dicloxacillin Oral Suspension contains not less than 90.0 per cent and not more than 120.0 per cent of the labeled amount of dicloxacillin, $C_{19}H_{17}Cl_2N_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of dicloxacillin, $C_{19}H_{17}Cl_2N_3O_5S$.

Usual strength. 12.5 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.7 to 7.5, of the constituted oral suspension.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.1 g.

Other tests. Comply with the tests stated under Oral Suspension.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Buffer solution. Dissolve 5.44 g of *monobasic potassium phosphate* in 2000 ml of *water*, adjusted to pH 5.0 with *8 M potassium hydroxide*.

Test solution. Transfer an acurately weighted quantity containing about 125 mg of dicloxacillin to a 200-ml flask. Add 20.0 ml of *dimethylformamide* and 5.0 ml of *ethanol*, and stir for 15 minutes. Add another 50.0 ml of buffer, sonicate this mixture for 15 minutes, dilute to 200.0 ml with the buffer solution and filter.

Reference solution. Weigh a 65 mg of *dicloxacillin sodium IPRS* in 100-ml volumetric flask, add 20.0 ml of *dimethylformamide*, 5.0 ml of *ethanol (95 per cent)*, and 20.0 ml of buffer solution, and stir for 5.0 minutes, dilute to volume with the buffer solution.

Chromatographic system

- a stainless steel column 30 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of the buffer solution and 25 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Inject the reference solution The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

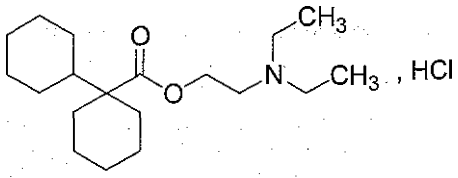
Inject the reference solution and the test solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$ in suspension.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Dicyclomine Hydrochloride

Dicycloverine Hydrochloride



$C_{19}H_{35}NO_2,HCl$ Mol. Wt. 346.0

Dicyclomine Hydrochloride is 2-diethylaminoethyl bicyclohexyl-1-carboxylate hydrochloride.

Dicyclomine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{19}H_{35}NO_2,HCl$, calculated on the dried basis.

Category. Antispasmodic.

Description. A white or almost white, crystalline powder.

Identification

A. Dissolve a suitable quantity in *acetone* and evaporate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicyclomine hydrochloride IPRS* or with the reference spectrum of dicyclomine hydrochloride.

B. To 3 ml of a 0.1 per cent w/v solution of *sodium dodecyl sulphate*, add 5 ml of *chloroform* and 0.05 ml of a 0.25 per cent w/v solution of *methylene blue*, mix gently and allow to separate; the chloroform layer is blue. Add 20 mg of the substance under examination dissolved in 2 ml of *water*, mix gently and allow to separate; the aqueous layer is blue and the chloroform layer is colourless.

C. Dissolve 10 mg in 5 ml of *water* and add 0.2 ml of *2 M nitric acid* and 0.5 ml of *silver nitrate solution*; a white precipitate is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *1-propanol*, 30 volumes of *ethyl acetate*, 15 volumes of *water* and 5 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.5 g of the substance under examination in 10 ml of *methanol*.

Reference solution. Dilute 5.0 ml of the test solution to 50.0 ml with *methanol* and mix. To 2.0 ml of the solution add sufficient *methanol* to produce 100.0 ml.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.6 g in 20 ml of *anhydrous glacial acetic acid* and add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03460 g of $C_{19}H_{35}NO_2,HCl$.

Storage. Store protected from moisture.

Dicyclomine Injection

Dicyclomine Hydrochloride Injection; Dicycloverine Hydrochloride Injection

Dicyclomine Injection is a sterile, isotonic solution of Dicyclomine Hydrochloride in Water for Injections.

Dicyclomine Injection contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of dicyclomine hydrochloride, $C_{19}H_{35}NO_2,HCl$.

Usual strength. 10 mg per ml.

Identification

A. To a volume containing 0.1 g of Dicyclomine Hydrochloride add 10 ml of *water* and 1 ml of *hydrochloric acid*, shake with 25 ml of *ether* and allow to separate. Extract the aqueous layer with 30 ml of *chloroform*, wash the extract with two quantities, each of 10 ml, of *water* and filter the chloroform solution through *anhydrous sodium sulphate*. Evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicyclomine hydrochloride IPRS* treated in the same manner or with the reference spectrum of dicyclomine hydrochloride.

B. In the Assay the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Bacterial endotoxins (2.2.3). Not more than 17.2 Endotoxin Unit per mg of Dicyclomine Hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 1 volume of *0.04 M phosphate buffer, pH 7.5* and 1 volume of *acetonitrile*.

Test solution. Dilute a volume containing about 20 mg of Dicyclomine Hydrochloride to 50.0 ml with the solvent mixture.

Reference solution. A 0.04 per cent w/v solution of *dicyclomine hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *0.02 M phosphate buffer pH 7.5* prepared by dissolving 2.72 g of *monobasic potassium phosphate* in 900 ml of *water*, adjusted to pH 7.5 with 10 per cent w/v solution of *sodium hydroxide*, diluting to 1000 ml with *water* and 70 volumes of *acetonitrile*,

- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{35}NO_2$,HCl in the injection.

Storage. Store protected from light, in single dose or multiple-dose containers.

Dicyclomine Oral Solution

Dicyclomine Hydrochloride Oral Solution; Dicycloverine Hydrochloride Oral Solution

Dicyclomine Oral Solution is a solution of Dicyclomine Hydrochloride in a suitable flavoured vehicle.

Dicyclomine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dicyclomine hydrochloride, $C_{19}H_{35}NO_2$,HCl.

Usual strength. 10 mg in 5 ml.

Identification

A. To a volume containing 0.1 g of Dicyclomine Hydrochloride add 10 ml of *water* and 1 ml of *hydrochloric acid*, shake with 30 ml of *ether* and allow to separate. Extract the aqueous layer with 30 ml of *chloroform*, wash the extract with two quantities, each of 10 ml, of *water* and filter the chloroform solution through *anhydrous sodium sulphate*. Evaporate the filtrate to dryness, recrystallise the residue from hot *acetone* and dry at 105° for 30 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicyclomine hydrochloride IPRS* or with the reference spectrum of dicyclomine hydrochloride.

B. Acidify the oral solution with *2 M nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

Tests

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Weigh a quantity containing about 5 mg of Dicyclomine Hydrochloride add 5 ml of *sulphuric acid (10 per cent v/v)* and 2 ml of *0.02 M potassium permanganate*, mix, allow to stand, add 20 ml of *water* and 20 ml of *chloroform* to the decolorised solution and titrate with *0.001 M sodium dodecyl sulphate*, using 1 ml of *dimethyl yellow solution* as indicator. 1 ml of *0.001 M sodium dodecyl sulphate* is equivalent to 0.0003460 g of $C_{19}H_{35}NO_2$,HCl.

Determine the weight per ml of the oral solution (2.4.29), and calculate the content of $C_{19}H_{35}NO_2$,HCl, weight in volume.

Storage. Store protected from light.

Dicyclomine Tablets

Dicyclomine Hydrochloride Tablets; Dicycloverine Hydrochloride Tablets

Dicyclomine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dicyclomine hydrochloride, $C_{19}H_{35}NO_2$,HCl.

Usual strength. 20 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.2 g of Dicyclomine Hydrochloride with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness, recrystallise the residue from hot *acetone* and dry at 105° for 4 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dicyclomine hydrochloride IPRS* or with the reference spectrum of dicyclomine hydrochloride.

B. To 3 ml of a 0.1 per cent w/v solution of *sodium dodecyl sulphate*, add 5 ml of *chloroform* and 0.05 ml of a 0.25 per cent w/v solution of *methylene blue*, mix gently and allow to separate; the chloroform layer is blue. Add about 20 mg of extracted powder obtained in identification test A, dispersed in 2 ml of *water*, mix gently and allow to separate; the aqueous layer is blue and the chloroform layer is colourless.

C. Shake a quantity of the powdered tablets containing 10 mg of Dicyclomine Hydrochloride with 5 ml of *water* and 0.2 ml of *2 M nitric acid*, filter and add 0.5 ml of *silver nitrate solution* to the filtrate; a white precipitate is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of buffer solution.

Test solution. Use the filtrate, dilute if necessary, with the solvent mixture.

Reference solution. Dissolve a quantity of *dicyclomine hydrochloride IPRS* in the solvent mixture, and dilute with the solvent mixture to obtain a solution having similar concentration to the test solution.

- injection volume: 250 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{35}NO_2.HCl$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{19}H_{35}NO_2.HCl$.

Limit of Dicyclomine related compound A. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 2.72 g of *monobasic potassium phosphate* in 900 ml of *water*, adjusted to pH 3.5 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Solvent mixture. 70 volumes of *acetonitrile* and 30 volumes of *water*.

Test solution. Disperse a quantity of powdered tablets containing 200 mg of Dicyclomine Hydrochloride in 10 ml of *water*, with the aid of ultrasound, add 70 ml of *acetonitrile*, sonicate for at least 5 minutes, and shake by mechanical means for at least 30 minutes and dilute to 100.0 ml with *water*. Centrifuge and use the clear supernatant.

Reference solution (a). A 0.0004 per cent w/v solution of *dicyclomine related compound A ([1,12 -Bi(cyclohexane)]-1-carboxylic acid) IPRS* in the solvent mixture.

Reference solution (b). Dilute 5.0 of reference solution (a) to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (3.5µm),
- mobile phase: A. 55 volumes of *acetonitrile* and 45 volumes of the buffer solution,
  B. 80 volumes of *acetonitrile* and 20 volumes of the buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
20	100	0
20.1	0	100
40	0	100
40.1	100	0
50	100	0

Inject reference solution (a) and (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (a) and signal-to-noise ratio is not less than 10 in the chromatogram obtained with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to dicyclomine related compound A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 2.72 g of *monobasic potassium phosphate* in 900 ml of *water*, adjusted to pH 7.5 with 10 per cent w/v solution of *sodium hydroxide* and dilute to 1000 ml with *water*.

Solvent mixture. 70 volumes of *acetonitrile* and 30 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 20 mg of Dicyclomine Hydrochloride in 2 ml of *water*, with the aid of ultrasound, add 35 ml of *acetonitrile*, sonicate for at least 5 minutes, and shake by mechanical means for at least 30 minutes and dilute to 50.0 ml with *water*. Centrifuge and use the clear supernatant.

Reference solution. A 0.04 per cent w/v solution of *dicyclomine hydrochloride IPRS* in the solvent mixture

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (3.5µm),
- mobile phase: a mixture of 70 volumes of *acetonitrile* and 30 volumes of buffer solution,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

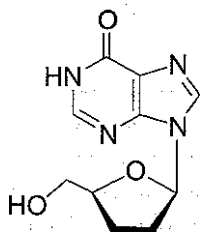
Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{35}NO_2$,HCl in the tablets.

Storage. Store protected from light.

Didanosine



$C_{10}H_{12}N_4O_3$ Mol. Wt. 236.2

Didanosine is 2′,3′–dideoxyinosine.

Didanosine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{10}H_{12}N_4O_3$, calculated on the dried basis.

Category. Antiretroviral.

Description. A white or almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *didanosine IPRS* or with the reference spectrum of didanosine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). –28.0° to –24.0°, determined in a 1.0 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.001 per cent w/v solution of the substance under examination in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 6 volumes of *acetonitrile* and 94 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than half of the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

Test solution. Dilute 5.0 ml of a 0.1 per cent w/v solution of the substance under examination in *water* to 100.0 ml with the mobile phase.

Reference solution. Dilute 5.0 ml of a 0.1 per cent w/v solution of *didanosine IPRS* in *water* to 100.0 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the column efficiency determined from the didanosine peak is not less than 5000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{12}N_4O_3$.

Storage. Store protected from light.

Didanosine Gastro-resistant Capsules

Didanosine Capsules

Didanosine Gastro-resistant Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of didanosine, $C_{10}H_{12}N_4O_3$. They are made gastro-resistant by enteric coating or by other means.

Usual strengths. 125 mg; 200 mg; 250 mg; 400 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

A. Apparatus No. 1 (Basket),

Medium. 1000 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 120 minutes.

Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 1.41 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml of *water*, adjusted to pH 7.5 with *orthophosphoric acid* and filter.

Test solution. Withdraw the medium completely without any loss of residue. Transfer the residue into 1000-ml volumetric flask, add 750 ml of the buffer solution, dissolve and make up the volume with the buffer solution. Dilute suitably with the buffer solution to get a solution containing 0.005 per cent w/v solution of Didanosine.

Reference solution. A 0.005 per cent w/v solution of *didanosine IPRS* in the buffer solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 95 volumes of the buffer solution and 5 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 249 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 1.5 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{12}N_4O_3$ released in the acid medium by subtracting the content of $C_{10}H_{12}N_4O_3$ in the test solution from the total content of Didanosine, $C_{10}H_{12}N_4O_3$ determined in the Assay.

Complies with the acceptance criteria given under acid stage.

B. Apparatus No. 1 (Basket),

Medium. 1000 ml of a buffer solution pH 6.8 prepared by mixing 25 volumes of *0.2 M tribasic sodium phosphate buffer* and 75 volumes of *0.1 M hydrochloric acid,* adjusted to pH 6.8 with *2 M hydrochloric acid* or *2 M sodium hydroxide,*

Speed and time. 100 rpm and 45 minutes.

Determine by liquid chromatography (2.4.14).

Transfer another 6 capsules and run the apparatus for 2 hours in *0.1 M hydrochloric acid*. Decant the medium without losing the residue, add buffer solution pH 6.8 and run the apparatus for 45 minutes. Withdraw a suitable volume of the medium and filter.

Test solution. Use the filtrate, dilute if necessary, to get a concentration of 0.005 per cent w/v of Didanosine in the dissolution medium.

Reference solution. A 0.005 per cent w/v solution of *didanosine IPRS* in the dissolution medium.

Use chromatographic system as described under Acid stage dissolution.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{12}N_4O_3$ in the medium

Q. Not less than 75 per cent of the stated amount of $C_{10}H_{12}N_4O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh a quantity of the contents of the capsules containing 100 mg of Didanosine, dissolve in 100.0 ml of mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of *didanosine IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5µm), (Such as Lichrospher RP18e),
- mobile phase: a mixture of 95 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water*, and 5 volumes of *acetonitrile*, adjusted to pH 6.8 with *triethylamine* and filter,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 4500 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 4 times the area of the peak in the chromatogram obtained with the reference solution (b) (4.0 per cent) and the sum of all the secondary peaks is not more than 5.5 times the area of the peak in the chromatogram obtained with the reference solution (5.5 per cent),

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the contents of the capsules containing 50 mg of Didanosine, dissolve in 100.0 ml of the *buffer solution pH 7.5* and filter. Dilute 5.0 ml of the solution to 50.0 ml with the *buffer solution pH 7.5*.

Reference solution. A 0.005 per cent w/v solution of *didanosine IPRS* in *buffer solution pH 7.5*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 950 volumes of *buffer solution pH 7.5* and 50 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 249 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{12}N_4O_3$ in the capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Didanosine Tablets

Didanosine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of didanosine, $C_{10}H_{12}N_4O_3$. The tablets may contain permitted flavouring agents.

Usual strengths. 25 mg; 50 mg;100 mg; 150 mg; 200 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Didanosine with 80 ml of *water*, dilute to 100 ml with *water* and filter. Dilute 5 ml of the filtrate to 100 ml with *water*. When examined in the range 220 nm to 350 nm (2.4.7), the resulting solution shows an absorption maximum only at about 250 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *didanosine IPRS* in dissolution medium to obtain a solution having a concentration similar to that of the test solution.

Chromatographic system
- a stainless steel column 12.5 cm × 4 mm, packed with octylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 95 volumes of 0.077 per cent w/v solution of *ammonium acetate* and 5 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: equivalent of 2 µg of didanosine.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{12}N_4O_3$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{10}H_{12}N_4O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the powdered tablets containing about 50 mg of Didanosine and transfer to a 50-ml volumetric flask. Add about 25 ml of *buffer solution pH 7.0*, and mix with the aid of ultrasound for 5 minutes, dilute to volume with the same solvent, mix and filter.

Reference solution. Weigh 50 mg *didanosine IPRS* and transfer to a 50-ml volumetric flask. Dissolve in about 25 ml of *buffer solution pH 7.0* and dilute to volume with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with the same solvent. Dilute further 5.0 ml to 50.0 ml with the same solvent and filter through a membrane filter disc with an average pore diameter not greater than 0.45 µm.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Kromasil C18),
- mobile phase: A. buffer solution pH 7.0 prepared by dissolving 1.42 g of *disodium hydrogen phosphate* and 6.8 g of *tetrabutylammonium hydrogen sulphate* in 1000 ml of *water*, adjusted to pH 7.0 with *sodium hydroxide solution*,

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
8	0	100
20	70	30
25	30	70
26	100	0
35	0	100
37	100	0

Inject the reference solution. The test is not valid unless the column efficiency determined from the didanosine peak is not less than 3000 theoretical plates and the tailing factor is not more than 1.5.

Inject separately the buffer and test solution. Examine the chromatogram obtained with the buffer solution for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 5.0 per cent and the sum of the areas of all the secondary peaks should not be more than 6.0 per cent when calculated by percentage area normalisation.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powdered tablets containing about 100 mg of Didanosine and transfer to a 100-ml volumetric flask. Add about 50 ml of *buffer solution pH 7.0*, mix with the aid of ultrasound for 10 minutes, dilute to volume with the same solvent, mix and filter through a membrane filter disc with an average pore diameter not greater than 0.45 µm.

Reference solution. A 0.1 per cent w/v solution of *didanosine IPRS* in *buffer solution pH 7.0*. Filter through a membrane filter disc with an average pore diameter not greater than 0.45 µm.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Kromasil C18),
- mobile phase: a filtered and degassed mixture of 5 volumes of *acetonitrile* and 95 volumes of a buffer solution prepared by dissolving 1.42 g of *disodium hydrogen phosphate* in 1000 ml of *water*, adjusted to pH 7.5 ± 0.05 with *dilute phosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 5 µl.

Inject the reference solution and run the chromatogram twice the retention time of didanosine. The test is not valid unless the column efficiency is not less than 4500 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

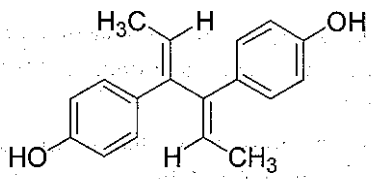
Calculate the content of $C_{10}H_{12}N_4O_3$ in the tablets.

Storage. Store protected from light.

Labelling. The label states that the tablets should be chewed before swallowing.

Dienoestrol

Dienestrol



$C_{18}H_{18}O_2$ Mol. Wt. 266.3

Dienoestrol is *(E,E)*-4,4'-[bis(ethylidene)ethylene]diphenol.

Dienoestrol contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{18}H_{18}O_2$, calculated on the dried basis.

Category. Oestrogen.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dienoestrol IPRS* or with the reference spectrum of dienoestrol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (a).

C. Heat a mixture of about 1 mg in 5 ml of *glacial acetic acid* and 1 ml of a 1 per cent w/v solution of *bromine* in *glacial acetic acid* in a water-bath for 2 minutes. To 0.5 ml of the solution in a dry test tube add 0.5 ml of *ethanol*, mix and add 10 ml of *water*; a reddish-violet colour is produced. Add 5 ml of *chloroform*, shake vigorously and allow to separate; the chloroform layer is red and the aqueous layer is almost colourless.

D. Dissolve 0.5 mg in 0.2 ml of *glacial acetic acid*, add 1 ml of *phosphoric acid* and heat on a water-bath for 3 minutes; a reddish-violet colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *diethylamine*.

Test solution (a). Dissolve 0.5 g of the substance under examination in 5 ml of *ethanol (95 per cent)*.

Test solution (b). Dilute 5 ml of test solution (a) to 100 ml with *ethanol (95 per cent)*.

Reference solution (a). A 0.5 per cent w/v solution of *dienoestrol IPRS* in *ethanol (95 per cent)*.

Reference solution (b). Dilute 5 ml of reference solution (a) to 50 ml with *ethanol (95 per cent)*.

Reference solution (c). A solution containing 0.25 per cent w/v each of *dienoestrol IPRS* and *stilbestrol IPRS* in *ethanol (95 per cent)*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent v/v)* and heat at 120° for 10 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows at least two clearly separated spots having approximately the same intensity.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 25 mg in sufficient *ethanol* to produce 100.0 ml. To 5.0 ml of the solution add 10 ml of *ethanol*, dilute with *0.1 M sodium hydroxide* to 250.0 ml and measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of $C_{18}H_{18}O_2$ from the absorbance obtained by repeating the procedure using *dienoestrol IPRS* in place of the substance under examination.

Storage. Store protected from light.

Dienoestrol Tablets

Dienestrol Tablets

Dienoestrol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dienoestrol, $C_{18}H_{18}O_2$.

Usual strength. 1 mg.

Identification

A. Extract a quantity of the powdered tablets containing about 15 mg of Dienoestrol with *ether* and filter; evaporate the filtrate to dryness. Reserve a portion of the residue for test C. Heat a mixture of about 1 mg of the residue in 5 ml of *glacial acetic acid* and 1 ml of a 1 per cent w/v solution of *bromine* in *glacial acetic acid* in a water-bath for 2 minutes. To 0.5 ml of the solution in a dry test tube add 0.5 ml of *ethanol*, mix and add 10 ml of *water*; a reddish-violet colour is produced. Add 5 ml of *chloroform*, shake vigorously and allow to separate; the chloroform layer is red and the aqueous layer is almost colourless.

B. Dissolve 0.5 mg of the residue in 0.2 ml of *glacial acetic acid*, add 1 ml of *phosphoric acid* and heat on a water-bath for 3 minutes; a reddish-violet colour is produced.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *diethylamine*.

Test solution. Shake a quantity of the powdered tablets containing 2 mg of Dienoestrol with 4 ml of *acetone*, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.05 per cent w/v solution of *dienoestrol IPRS* in *acetone*.

Reference solution (b). A solution containing 0.1 per cent w/v each of *dienoestrol IPRS* and *stilbestrol IPRS* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent v/v)* and heat at 120° for 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots having approximately the same intensity.

Tests

Uniformity of content. Complies with test stated under Tablets.

Powder one tablet and extract with successive quantities of *ether* until complete extraction is effected. Filter the ether solution and wash the filter paper with small quantities of *ether*. Evaporate the *ether* and dissolve the residue in 10 ml of *ethanol* and add sufficient *0.1 M sodium hydroxide* to produce a solution containing 0.0005 per cent w/v of Dienoestrol. Complete the test as described under Assay beginning at the words "Measure the absorbance....".

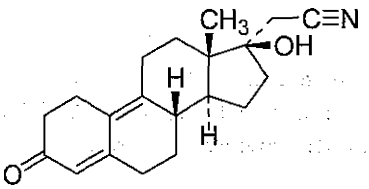
Calculate the content of $C_{18}H_{18}O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 10 mg of Dienoestrol and triturate with successive quantities of *ether* until complete extraction is effected. Filter the ether extracts and wash the filter with small quantities of *ether*. Combine the filtrate and washings and remove the *ether*; dissolve the residue in sufficient *ethanol* to produce 50.0 ml. To 5.0 ml of the solution add 10 ml of *ethanol* and sufficient *0.1 M sodium hydroxide* to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at 245 nm (2.4.7). Calculate the content of $C_{18}H_{18}O_2$ from the absorbance obtained by repeating the operation using a solution obtained by dissolving 2.5 mg, weighed, of *dienoestrol IPRS* in 20 ml of *ethanol* and diluting with sufficient *0.1 M sodium hydroxide* to produce 500.0 ml.

Storage. Store protected from light.

Dienogest



$C_{20}H_{25}NO_2$ Mol Wt. 311.4

Dienogest is 2-[(8*S*, 13 *S*, 14 *S*, 17*R*)-17-hydroxy-13-methyl-3-oxo-1,2,6,7,8,11,12,14,15,16-decahydrocyclopenta phenanthren-17-yl]acetonitrile.

Dienogest contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{25}NO_2$, calculated on the dried basis.

Category. Antiandrogenic.

Description. An off-white to slightly yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dienogest IPRS* or with the reference spectrum of dienogest.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 60 ml of the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.025 per cent w/v solution of *dienogest IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water* and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.2 per cent and the sum of the area of all secondary peaks is not more than 0.5 per cent, calculated by area normalization.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 60 ml of the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.025 per cent w/v solution of *dienogest IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

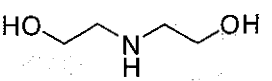
Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}NO_2$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Diethanolamine



$C_4H_{11}NO_2$ Mol. Wt. 105.14

Diethanolamine is bis(hydroxyethyl)amine.

Diethanolamine is a mixture of ethanolamines, consisting largely of diethanolamine.

Diethanolamine contains not less than 98.5 per cent and not more than 101.0 per cent of ethanolamines, $NH(C_2H_4OH)_2$, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethanolamine IPRS* or with the reference spectrum of diethanolamine.

Tests

Refractive index (2.4.27). 1.473 to 1.476, determined at 30°.

Triethanolamine. Not more than 1.0 per cent.

To 100 ml of *methanol*, add 6 to 8 drops of mixed indicator of 0.15 g of *methyl orange* and 0.08 g of *xylene cyanole* in 100 ml of *water* in a 500-ml glass-stoppered conical flask and neutralize with *0.1 M ethanolic sulphuric acid* or *0.1 M ethanolic potassium hydroxide*. The neutral solution is amber when viewed by transmitted light and is red-brown when viewed by reflected light, add 20 g of the substance under examination, add into 500-ml glass-stoppered conical flask, cautiously add 75 ml of *acetic anhydride*, and swirl to effect complete solution. Allow to stand at room temperature for 30 minutes, cool. Titrate with *0.5 M ethanolic sulphuric acid solution*. Carry out a blank titration.

1 ml of *0.5 M ethanolic sulphuric acid* is equivalent to 0.0746 g of triethanolamine.

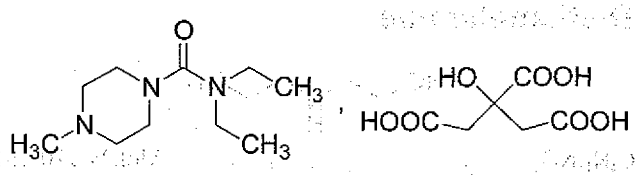
Water (2.3.43). Not more than 0.15 per cent, determined on a 20.0 g of substance under examination in a mixture of 25 volumes of *glacial acetic acid* and 40 volumes of *methanol*.

Assay. Dissolve 2.0 g in 50.0 ml of *water*. Titrate with *0.5 M hydrochloric acid* using *bromocresol green solution* as indicator. Carry out a blank titration.

1 ml of *0.5 M hydrochloric acid* is equivalent to 0.05257 g of $NH(C_2H_4OH)_2$.

Storage. Store protected from light and moisture.

Diethylcarbamazine Citrate



$C_{10}H_{21}N_3O,C_6H_8O_7$ Mol. Wt. 391.4

Diethylcarbamazine Citrate is *N,N*-diethyl-4- methylpiperazine-1-carboxamide dihydrogen citrate.

Diethylcarbamazine Citrate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{10}H_{21}N_3O,C_6H_8O_7$, calculated on the dried basis.

Category. Anthelmintic; antifilarial.

Description. A white, crystalline powder; slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethylcarbamazine citrate IPRS* or with the reference spectrum of diethylcarbamazine citrate.

B. In the test for *N,N'*-Dimethylpiperazine and *N*-methylpiperazine, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

C. A 2 per cent w/v solution gives reactions of citrates (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

***N,N'*-Dimethylpiperazine and *N*-methylpiperazine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 65 volumes of *methanol*, 30 volumes of *2-butanone* and 5 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.5 g of the substance under examination in 10 ml of *methanol*.

Reference solution (a). A 5 per cent w/v solution of *diethylcarbamazine citrate IPRS* in *methanol*.

Reference solution (b). A 0.01 per cent w/v solution of *N,N'- dimethylpiperazine* in *methanol*.

Reference solution (c). A 0.01 per cent w/v solution of *N-methylpiperazine* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 105° and expose it to iodine vapour for 30 minutes. Any spots corresponding to *N,N'*-dimethylpiperazine and *N*-methylpiperazine in the chromatogram obtained with the test solution are not more intense than the spots in the chromatogram obtained with reference solution (b) and (c) respectively.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 31.2 g of *potassium dihydrogen phosphate* in *water* and dilute to 1000 ml with the same solvent.

Test solution. Suspend 0.3 g of the substance under examination in the solvent mixture and dilute to 100 ml with the same solvent, filter and use the supernatant.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with solvent mixture.

Reference solution (b). To 3.0 ml of the test solution, add 0.5 ml of *strong hydrogen peroxide solution* and maintain at 80° for 3 hours. Dilute to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *methanol* and 90 volumes of a 1.0 per cent w/v solution of *potassium dihydrogen phosphate,*
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

The relative retention time with reference to diethylcarbamazine for citrate is about 0.2 and for degradation product is about 1.6 and the retention time of diethylcarbamazine is about 7 minutes.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to diethylcarbamazine and the degradation product is not less than 5.0.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent). Ignore the peak due to citrate.

Heavy metals (2.3.13). 1.0 g dissolved in 20 ml of *water*, 0.5 ml of *0.1 M hydrochloric acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure of 1.5 to 2.5 kPa for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 25 mg of the substance under examination, dissolve in 20 ml of a 3.124 per cent w/v solution of *potassium dihydrogen phosphate*, dilute to 25.0 ml with the same solvent, mix well and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the potassium dihydrogen phosphate solution.

Reference solution. A 0.01 per cent w/v solution of *diethylcarbamazine citrate IPRS* in a 3.124 per cent w/v solution of *potassium dihydrogen phosphate.*

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *methanol* and 90 volumes of a 1 per cent solution of *potassium dihydrogen phosphate,*
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{21}N_3O,C_6H_8O_7$.

Storage. Store protected from moisture.

Diethylcarbamazine Tablets

Diethylcarbamazine Citrate Tablets

Diethylcarbamazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of diethylcarbamazine citrate, $C_{10}H_{21}N_3O,C_6H_8O_7$.

Usual strengths. 50 mg; 100 mg.

Identification

To a quantity of the powdered tablets containing 0.15 g of Diethylcarbamazine Citrate add 15 ml of *ethanol (95 per cent)*, shake for 5 minutes, filter and evaporate the filtrate to dryness. To the residue add 10 ml of *2 M sodium hydroxide* and extract with three quantities, each of 10 ml, of *chloroform.* Dry the combined extracts over *anhydrous sodium sulphate*, filter and evaporate the *chloroform.* The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethylcarbamazine citrate IPRS* treated in the same manner or with the reference spectrum of diethylcarbamazine.

Tests

***N,N'*-Dimethylpiperazine and *N*-methylpiperazine.** Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 65 volumes of *methanol*, 30 volumes of *2-butanone* and 5 volumes of *strong ammonia solution*.

Test solution. Disperse a quantity of powdered tablets containing 0.5 g of the diethylcarbamazine citrate in *methanol* and dilute to 10.0 ml with *methanol*, filter.

Reference solution (a). A 0.01 per cent w/v solution of *N,N'-dimethylpiperazine* in *methanol*.

Reference solution (b). A 0.01 per cent w/v solution of *N-methylpiperazine* in *methanol*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 105° and expose it to iodine vapour for 30 minutes. Any spots corresponding to *N,N'*-dimethylpiperazine and *N*-methylpiperazine in the chromatogram obtained with the test solution are not more intense than the spots in the chromatogram obtained with reference solution (a) and (b) respectively.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute the filtrate, if necessary, with an equal volume of a 6.248 per cent w/v solution of *potassium dihydrogen phosphate*. Carry out the determination as described under Assay. Calculate the content of $C_{10}H_{21}N_3O,C_6H_8O_7$ using a solution of known concentration of *diethylcarbamazine citrate IPRS* in a 3.124 per cent w/v solution of *potassium dihydrogen phosphate*.

Q. Not less than 75 per cent of the stated amount of $C_{10}H_{21}N_3O,C_6H_8O_7$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 25 mg of Diethylcarbamazine Citrate, add 20 ml of a 3.124 per cent w/v solution of *potassium dihydrogen phosphate* and place in an ultrasonic bath for 5 minutes. Cool, dilute to 25.0 ml with the same solvent and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the same solvent.

Reference solution. A 0.01 per cent w/v solution of *diethylcarbamazine citrate IPRS* in a 3.124 per cent w/v solution of *potassium dihydrogen phosphate*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *methanol* and 90 volumes of a 1 per cent w/v solution of *potassium dihydrogen phosphate*,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

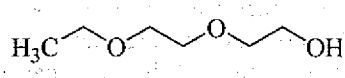
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{21}N_3O,C_6H_8O_7$ in the tablets.

Storage. Store protected from moisture.

Diethylene Glycol Monoethyl Ether



$C_6H_{14}O_3$ Mol. Wt. 134.2

Diethylene Glycol Monoethyl Ether contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_{14}O_3$, calculated on the anhydrous basis.

Category. Pharmaceutical aid (humectant; solvent).

Description. A clear, colourless liquid , hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethylene glycol monoethyl ether IPRS* or with the reference spectrum of diethylene glycol monoethyl ether.

B. In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Acid value (2.3.23). Not more than 0.1.

Peroxide value (2.3.35). Not more than 8.0.

Refractive index (2.4.27). 1.426 to 1.428 at 20°.

Related substances. Determine by gas chromatography (2.4.13).

Free ethylene oxide. Not more than 1µg per g.

CAUTION— Ethylene oxide is toxic and flammable. Prepare these solutions in a well ventilled fume hood, using great care. Protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask.

NOTE— Prepare the solutions immediately before use.

Acetaldehyde solution. A 0.001 per cent v/v solution of *acetaldehyde* in *water*.

Test solution (a). Fill a chilled pressure bottle with liquid *ethylene oxide*, and store in a freezer when not in use. Use a small piece of polyethylene film to protect the liquid from contact with the rubber gasket. Tare a glass stoppered conical flask, add 50 ml of *polyethylene glycol 200* and reweigh the flask. Transfer 5 ml of the liquid *ethylene oxide* into a 100 ml beaker chilled in a mixture of one volume *sodium chloride* and three volumes wet ice. Using a syringe that has been previously cooled to -10°, transfer 300 µl (equivalent to about 250 mg) of liquid *ethylene oxide* to the *polyethylene glycol 200*, and swirl gently to mix. Replace the stopper, reweigh the flask and determine the amount of *ethylene oxide* absorbed by weigh difference. Adjust the weight of the mixture with *polyethylene glycol 200* to 100 g, replace the stopper and swirl gently to mix. This solution containing 2.5 mg/g of *ethylene oxide*.

Test solution (b). Transfer 1 g *diethylene glycol monoethyl ether* to 10.0 ml pressure headspace vial add 1ml of *water*, seal the vial and mix. Heat the mixture at 70° for 45 minutes.

Reference solution (a). Tare a glass stoppered conical flask and chill it in a refrigerator. Add 35 ml of *polyethylene glycol 200* and reweigh the flask. Use a syringe that has been chilled in a refrigerator and transfer 1g of the chilled test solution (a) to the tared, conical flask. Adjust the weight of the solution with *polyethylene glycol 200* to 50 g, replace the stopper and swirl gently to mix. Transfer 10 g of the solution to a 50-ml volumetric flask. Add 30 ml of *water* and mix. Dilute with *water* to volume and mix to obtain a 0.001 per cent w/v solution of *ethylene oxide*.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 50.0 ml with *water* to produce 0.0002 per cent w/v solution of *ethylene oxide*.

Reference solution (c). Dilute 0.5 ml of reference solution (b) to 10.0 ml pressure headspace vial add 0.1 ml of *acetaldehyde solution* and 0.1 ml of *water*, seal the vial and mix. Heat the mixture at 70° for 45 minutes.

Reference solution (d). Transfer 1 g *diethylene glycol monoethyl ether* to 10.0 ml pressure headspace vial add 0.5 ml of reference solution (b) and 0.5 ml of *water*, seal the vial and mix. Heat the mixture at 70° for 45 minutes.

Chromatographic system

- a glass or quartz capillary column 30 m x 0.32 mm, bonded with a 1.0 µm layer of phase G1,
- temperature:

Column	Intial temperature (°)	Ramp temperature (°/min.)	Final temperature (°)	Hold time at final temperature (min.)
	50	—	50	5
	50	5	180	—
	180	30	230	5

- inlet port at 150° and detector at 250°,
- flame ionization detector,
- flow rate: 1.0 ml per minute, using helium or nitrogen as the carrier gas,
- injection volume: 1 ml.

Name	Relative retention time
acetaldehyde	0.94
ethylene oxide	1.0

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to acetaldehyde and ethylene oxide is not less than 2.0 and relative standard deviation for replicate injections is not more than 15 per cent.

Inject reference solution (d) and test solution (b). Calculate the amount of ethylene oxide in diethylene glycol monoethyl ether using the following equation.

$$\frac{r_u}{(r_s \times w_u) - (r_u \times w_s)}$$

where, r_u = ethylene oxide peak area from the test solution (b),

r_s = ethylene oxide peak area from the reference solution (d),

w_u = weigh of diethylene glycol monoethyl ether to prepare the test solution (b),

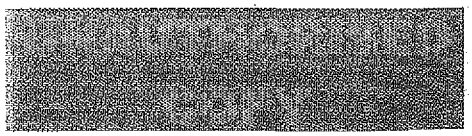
w_s = weigh of diethylene glycol monoethyl ether to prepare the reference solution (d).

Limit of 2-methoxyethanol, 2-ethoxyethanol, ethylene glycol and diethylene glycol

Name	Not more than (ppm)
2-methoxyethanol	50
2-ethoxyethanol	160
ethylene glycol	620
diethylene glycol	150

Use the test solution, reference solution, chromatographic system and system suitability requirements as described under Assay.

Inject the test solution.



Calculate the percentage of 2-methoxyethanol in diethylene glycol monoethyl ether using the following equation.

$$\frac{r_U}{r_T} \times 100$$

where, r_U = peak response for 2-methoxyethanol,
r_T = sum of the responses of all the peaks.

Calculate the percentage of 2-ethoxyethanol in diethylene glycol monoethyl ether using the following equation.

$$\frac{r_U}{r_T} \times 100$$

where, r_U = peak response for 2-ethoxyethanol,
r_T = sum of the responses of all the peaks.

Calculate the percentage of ethylene glycol in diethylene glycol monoethyl ether using the following equation.

$$\frac{r_U}{r_T} \times 100$$

where, r_U = peak response for ethylene glycol,
r_T = sum of the responses of all the peaks.

Calculate the percentage of diethylene glycol in the portion of diethylene glycol monoethyl ether using following equation.

$$\frac{r_U}{r_T} \times 100$$

where, r_U = peak response for diethylene glycol,
r_T = sum of the responses of all the peaks.

Water (2.3.43). Not more than 0.1 per cent, determined on 10.0 g.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Use the substance under examination .

Reference solution. Dissolve 100 mg of each of *2-methoxyethanol*, *2- ethoxyethanol*, *ethylene glycol*, *diethylene glycol* and *diethylene glycol monoethyl ether IPRS* in 100.0 ml of *methanol*.

Chromatographic system

- a fused silica column 30 m x 0.32 mm, bonded with a 1.0 µm layer of phase G 46,
- temperature:

Column	Intial temperature (°)	Ramp temperature (°/min.)	Final temperature (°)	Hold time at final temperature (min.)
	120	—	120	1
	120	12	225	2

- inlet port at 250° and detector at 275°,
- flame ionization detector,
- flow rate: 2.2 ml per minute, using helium or nitrogen as the carrier gas,
- injection volume: 0.5µl.

Name	Relative retention time
2- methoxyethanol	0.40
2- ethoxyethanol	0.43
ethylene glycol	0.50
diethylene glycol monoethyl ether	0.93
diethylene glycol	1.0

Inject the reference solution. The test is not valid unless the resolution between the peaks corresponding to 2-ethoxyethanol and ethylene glycol is not less than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

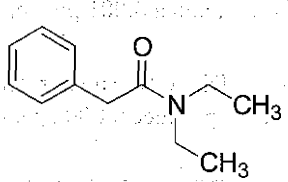
Inject the test solution. Calculate the percentage of diethylene glycol monoethyl ether using following equation.

$$\frac{r_U}{r_T} \times 100$$

where, r_U = peak response for diethylene glycol monoethyl ether,
r_T = sum of the responses of all the peaks

Storage. Store in well-closed containers under an atmosphere of inert gas, at a temperature not exceeding 35°.

Diethylphenylacetamide



$C_{12}H_{17}NO$ Mol. Wt. 191.3

Diethylphenylacetamide is *N,N*-diethylbenzeneacetamide

Diethylphenylacetamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{17}NO$, calculated on the anhydrous basis.

Category. Insect repellent.

Description. A clear to faintly yellow liquid. It shall be free from suspended matter.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Boiling point (2.4.8). About 290°.

Relative density (2.4.29). About 1.01 at 25°.

Refractive index (2.4.27). 1.520 to 1.521 at 20° sodium D lines.

Water (2.3.43). Not more than 0.5 per cent.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. A 3 per cent w/v solution of *diethyl sebacate* in *acetone*.

Test solution. To 0.2 g of the substance under examination, add 10 ml of internal standard solution and dilute to the 100.0 ml with *acetone*.

Reference solution. To 50 mg of *diethylphenylacetamide IPRS*, add 2.5 ml of internal standard solution and dilute to the 25.0 ml with *acetone*.

Chromatographic system

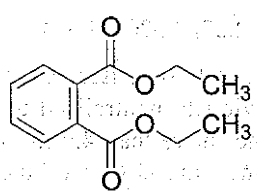
- a glass column 1.2 m x 2 mm packed with 10.0 per cent OV-101 on chromosorb WHP (100-120 mesh),
- temperature :
  column 150°,
  inlet port and detector at 300°,
- flow rate 30 ml per minute of the Nitrogen, 30 ml per minute of the Hydrogen, 210 ml per minute of the Air.

Inject 2 µl of the test solution and the reference solution.

Calculate the content of $C_{12}H_{17}NO$.

Storage. Store protected from light and moisture.

Diethyl Phthalate



$C_{12}H_{14}O_4$ Mol. Wt. 222.2

Diethyl phthalate is diethyl benzene-1,2-dicarboxylate.

Diethyl phthalate contains not less than 99.0 per cent and not more than 101.0 per cent of diethyl phthalate, $C_{12}H_{14}O_4$.

Category. Pharmaceutical aid.

Description. A clear, oily liquid, colourless or very slightly yellow.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethyl phthalate IPRS* or with the reference spectrum of diethyl phthalate.

B. Relative density (2.4.29). 1.117 to 1.121.

C. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 30 volumes of *heptane* and 70 volumes of *ether*.

Test solution. Dissolve 50 mg of the substance under examination in 10 ml of *ether*.

Reference solution. Dissolve 50 mg of *diethyl phthalate IPRS* in 10 ml of *ether*.

Apply to the plate 10 µl of each solution. After development dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

D. To about 0.1 ml, add 0.25 ml of *sulphuric acid* and 50 mg of *resorcinol*. Heat on a water-bath for 5 minutes. Allow to cool. Add 10 ml of *water* and 1 ml of *strong sodium hydroxide solution*. The solution becomes yellow or brownish-yellow and shows green fluorescence.

Tests

Appearance. The substance under examination is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

Acidity. Dissolve 20.0 g in 50 ml of *alcohol* previously neutralised to *phenolphthalein solution*. Add 0.2 ml of *phenolphthalein solution*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Related substances. Determine by gas chromatography (2.4.13).

Internal standard solution. Dissolve 60 mg of *naphthalene* in 20 ml of *methylene chloride*.

Test solution (a). Dissolve 1 g of the substance under examination in 20 ml of *methylene chloride*.

Test solution (b). Dissolve 1 g of the substance under examination in *methylene chloride*, add 2.0 ml of the internal standard solution and dilute to 20 ml with *methylene chloride*.

Reference solution. To 1 ml of test solution (a) add 10 ml of the internal standard solution and dilute to 100 ml with *methylene chloride*.

Chromatographic system
- a glass column 2.0 m x 2 mm, packed with silanised diatomaceous earth for gas chromatography (150 µm to 180 µm) impregnated with 3.0 per cent m/m of *polymethylphenylsiloxane*,
- temperature :
  column 150°,
  inlet port and detector at 225°,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks corresponding to naphthalene and diethyl phthalate is at least 10.

Inject 1 µl of test solution (a). In the chromatogram obtained, verify that there is no peak with the same retention time as the internal standard.

Inject separately 1 µl of test solution (b) and the reference solution. Continue the chromatography for three times the retention time of diethyl phthalate. From the chromatogram obtained with the reference solution, calculate the ratio (*R*) of the area of the peak due to diethyl phthalate to the area of the peak due to the internal standard. From the chromatogram obtained with test solution (b), calculate the ratio of the sum of the areas of all peaks, other than the principal peak and the peaks due to the internal standard and the solvent, to the area of the peak due to the internal standard; this ratio is not greater than *R* (1.0 per cent).

Water (2.3.43). Not more than 0.2 per cent, determined on 5.0 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 1.0 g.

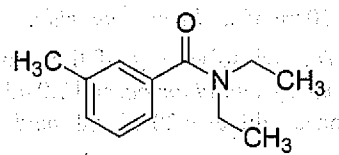
Assay. Dissolve 0.75 g in 25.0 ml of *0.5 M alcoholic potassium hydroxide* and add few glass beads. Boil on a water-bath under a reflux condenser for 1 hour. Add 1 ml of *phenolphthalein solution* and titrate immediately with *0.5 M hydrochloric acid*. Carry out a blank titration.

1 ml of *0.5 M alcoholic potassium hydroxide* is equivalent to 0.05556 g of $C_{12}H_{14}O_4$.

Storage. Store protected from moisture.

Diethyltoluamide

Deet



$C_{12}H_{17}NO$ Mol. Wt. 191.3

Diethyltoluamide is *N,N*-diethyl-*3*-toluamide.

Diethyltoluamide contains not less than 95.0 per cent and not more than 103.0 per cent of $C_{12}H_{17}NO$, calculated on the anhydrous basis.

Category. Insect repellent.

Description. A colourless or faintly yellow liquid.

CAUTION — Diethyltoluamide is irritant to the eyes and mucous membranes.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diethyltoluamide IPRS*.

B. Heat 2 ml with 25 ml of a 50 per cent v/v solution of *hydrochloric acid* under a reflux condenser for 1 hour. Make the mixture alkaline with *sodium hydroxide solution*, cool and extract with three quantities, each of 30 ml, of *ether*. Reserve the aqueous layer. Evaporate the ether, dissolve the residue in 5 ml of *dilute hydrochloric acid*, cool to 5°, add 5 ml of *sodium nitrite solution* and allow to stand for 10 minutes at 5°. Add 10 ml of *water* and extract with two quantities, each of 20 ml, of *ether*. Evaporate the ether, add 1 g of *phenol* to the residue, cool and add 1 ml of *sulphuric acid*; an intense green colour is produced, which becomes red on pouring into *water* and green on making alkaline with *dilute sodium hydroxide solution*.

C. Acidify the aqueous layer reserved in test B with *dilute hydrochloric acid*, extract with two quantities, each of 20 ml, of *ether* and evaporate the ether from the combined extracts. The residue, after drying at 60°, melts at about 108° (2.4.21).

Tests

Weight per ml (2.4.29). 0.997g to 1.000 g, determined at 20°.

Refractive index (2.4.27). 1.520 to 1.524.

Acidity. A solution of 10.0 g dissolved in 50 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution* requires not more than 4.0 ml of *0.01 M sodium hydroxide* to change the colour of the solution, using *phenolphthalein solution* as indicator.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

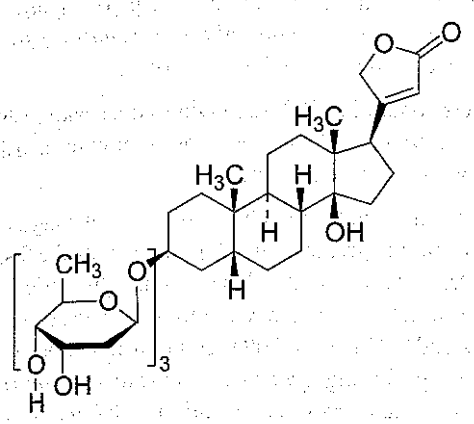
Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Weigh 0.3 g, add 7 ml of *nitrogen-free sulphuric acid* and carry out the determination of nitrogen (2.3.30), using *0.05 M sulphuric acid* as an absorbent solution or distill the liberated ammonia in 25 ml of *0.05 M sulphuric acid*.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.01913 g of $C_{12}H_{17}NO_5$.

Storage. Store protected from moisture in dry containers.

Digitoxin



$C_{41}H_{64}O_{13}$ Mol. Wt. 764.9

Digitoxin is 3β-[(*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-14β-hydroxy-5β-card-20(22)-enolide.

Digitoxin contains not less than 95.0 per cent and not more than 103.0 per cent of $C_{41}H_{64}O_{13}$, calculated on the dried basis.

Category. Cardiotonic.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry 2.4.6). Compare the spectrum with that obtained with *digitoxin IPRS* or with the reference spectrum of digitoxin.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. Dissolve about 1 mg in 2 ml of *glacial acetic acid* with the aid of gentle heat, cool and add 0.05 ml of *ferric chloride test solution*. Cautiously add 1 ml of *sulphuric acid* under the two liquids without mixing; a brown ring develops at the interface which gradually becomes blue and a green colour, finally passes to the upper layer.

D. Suspend about 0.5 mg in 0.2 ml of *ethanol (60 per cent)* and add 0.1 ml *dinitrobenzoic acid solution* and 0.1 ml of *2 M sodium hydroxide*; a violet colour develops.

Tests

Appearance of solution. A 0.5 per cent w/v solution in a mixture of equal volumes of *chloroform* and *methanol* is clear (2.4.1), and colourless (2.4.1).

Specific optical rotation (2.4.22). +16.0° to +18.5°, determined at 20° in a 2.5 per cent w/v solution in *chloroform*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 40 volumes of *cyclohexane* and 15 volumes of *methanol*.

Solvent mixture. A mixture of equal volumes of *chloroform* and *methanol*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

Reference solution (a). A 1.0 per cent w/v solution of *digitoxin IPRS* in the same solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the same solvent mixture.

Reference solution (c). A 0.02 per cent w/v solution of *gitoxin IPRS* in the same solvent mixture.

Reference solution (d). Dilute 5 ml of reference solution (b) to 10 ml with the same solvent mixture.

Reference solution (e). A solution containing 0.5 per cent w/v of *digitoxin IPRS* and 0.01 per cent w/v of *gitoxin IPRS* in the same solvent mixture.

Apply to the plate 5 µl of each solution and develop the chromatograms immediately after applying the solutions. After development, dry the plate in a current of cold air for 5 minutes. Repeat the development and again dry the plate in a current of cold air for 5 minutes. Spray with *ethanolic sulphuric acid (10 per cent)* and heat at 130° for 15 minutes. Examine the chromatograms in daylight. Any spot in the chromatogram obtained with the test solution corresponding to gitoxin is not more intense than the spot in the chromatogram obtained with reference solution (c). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (e) shows clearly separated spots corresponds to digitoxin and gitoxin and the spot in the chromatogram obtained with reference solution (d) is clearly visible.

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on the residue obtained from the test for Loss on drying.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 0.5 g by drying in an oven at 105° for 2 hours.

Assay. Dissolve 40 mg in sufficient *ethanol (95 per cent)* to produce 50.0 ml and dilute 5.0 ml of the solution to 100.0 ml with the same solvent. To 5.0 ml of the solution add 3.0 ml of *alkaline picric acid solution*, allow to stand in subdued light for 30 minutes and measure the absorbance of the resulting solution at the maximum at 495 nm (2.4.7), using as the blank a mixture of 5.0 ml of *ethanol (95 per cent)* and 3.0 ml of *alkaline picric acid solution*. Calculate the content of $C_{41}H_{64}O_{13}$ from the absorbance obtained by repeating the operation using *digitoxin IPRS* in place of the substance under examination.

Storage. Store protected from moisture and light in a refrigerator (2° to 8°).

Digitoxin Tablets

Digitoxin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of digitoxin, $C_{41}H_{64}O_{13}$.

Usual strengths. 100 µg; 200 µg.

Identification

To a quantity of the powdered tablets containing 250 µg of Digitoxin add 1 ml of *glacial acetic acid* containing 0.01 per cent w/v of *ferric chloride*, shake for a few minutes, filter through sintered-glass and add cautiously 1 ml of *sulphuric acid* to the filtrate without mixing; a brown ring free from red colour is produced at the interface which gradually becomes blue and finally the upper layer acquires an indigo colour.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 600 ml of freshly distilled *water*,
Speed and time. 120 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first 1 ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of *L-ascorbic acid* in *methanol* and 0.2 ml of a 0.009M solution of hydrogen peroxide [prepared by accurately diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with *0.02 M potassium permanganate*], mix and dilute to volume with *hydrochloric acid*. After exactly 30 minutes measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 400 nm and an emission wavelength of about 570 nm and setting the spectrophotofluorimeter to zero with *water* and to 100 with a solution of suitable concentration of *digitoxin IPRS* prepared at the same time and treated in the same manner as the test solution.

Q. Not less than 75 per cent of the stated amount of digitoxin, $C_{41}H_{64}O_{13}$.

Uniformity of content. Complies with test stated under Tablets.

Test solution. For tablets containing 100 µg of Digitoxin, shake 1 tablet with 15 ml of *methanol (50 per cent)* for 30 minutes and dilute to 25.0 ml with the same solvent.

For tablets containing 200 µg of Digitoxin, shake 1 tablet with 30 ml of *methanol (50 per cent)* for 30 minutes and dilute to 50.0 ml with the same solvent.

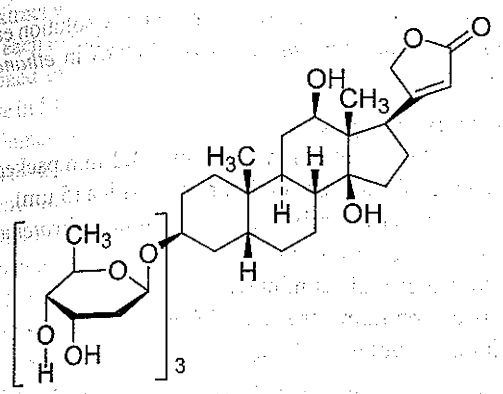
Filter through a suitable membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first few ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of *L-ascorbic acid* in *methanol* and 0.2 ml of a 0.009M solution of hydrogen peroxide [prepared by diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with *0.02 M potassium permanganate*], mix and dilute to volume with *hydrochloric acid*. After exactly 30 minutes measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 400 nm and an emission wavelength of about 570 nm and setting the spectrophotofluorimeter to zero with *water*. Calculate the content of digitoxin, $C_{41}H_{64}O_{13}$, from the *fluorescence* obtained by carrying out the operation described above at the same time using a 0.0004 per cent w/v solution of *digitoxin IPRS* in *methanol (50 per cent)* and beginning at the words "Transfer 1.0 ml to a 10-ml volumetric flask....".

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 1.25 mg of Digitoxin, add 3.0 ml of *water*, swirl to disperse the powder and allow to stand for 10 minutes, swirling occasionally. Add 25.0 ml of *glacial acetic acid*, shake for 1 hour and filter, discarding the first few ml of the filtrate. To 4.0 ml of the filtrate add 1.0 ml of *dimethyl sulphoxide, dilute* to 25.0 ml with *xanthydrol reagent*, mix well and allow to stand in the dark for 4 ½ hours (solution A). At the same time prepare two further solutions in the same manner but using for solution B 4.0 ml of *digitoxin standard solution* and for solution C 4.0 ml of a mixture of 25 volumes of *glacial acetic acid* and 3 volumes of *water* and beginning at the words "add 1.0 ml of *dimethyl sulphoxide*.....". Measure the absorbances of solutions A and B at the maximum at 550 nm (2.4.7), using solution C as the blank. Calculate the content of $C_{41}H_{64}O_{13}$ from the absorbances obtained.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Digoxin



$C_{41}H_{64}O_{14}$ Mol. Wt. 780.9

Digoxin is 3β-[(*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1→4)-*O*-2,6-dideoxy-β-D-*ribo*-hexopyranosyl-(1128W1Ä4)-2, 6-dideoxy-β-D-*ribo*-hexopyranosyl)oxy]-12b,1428β-dihydroxy-5β-card-20(22)-enolide.

Digoxin contains not less than 95.0 per cent and not more than 103.0 per cent of $C_{41}H_{64}O_{14}$, calculated on the dried basis.

Category. Cardiotonic.

Description. Colourless crystals or a white or almost white powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *digoxin IPRS* or with the reference spectrum of digoxin.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve about 1 mg in 2 ml of *glacial acetic acid* with the aid of gentle heat, cool and add 0.05 ml of *ferric chloride test solution*. Cautiously add 1 ml of *sulphuric acid* under the two liquids without mixing; a brown ring develops at the interface which gradually becomes blue and a green colour, finally passes to the upper layer.

D. Suspend about 0.5 mg in 0.2 ml of *ethanol (60 per cent)* and add 0.1 ml *dinitrobenzoic acid solution* and 0.1 ml of *2 M sodium hydroxide*; a violet colour develops.

Tests

Appearance of solution. A 0.5 per cent w/v solution in a mixture of equal volumes of *dichloromethane* and *methanol* is clear (2.4.1), and colourless (2.4.1).

Specific optical rotation (2.4.22). +10.0° to +13.0°, determined in a 2.0 per cent w/v solution in *anhydrous pyridine*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 50 mg of the substance under examination in the *methanol* and dilute to 100.0 ml with the *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *digoxin IPRS* in *methanol*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*.

Reference solution (c). A 0.0001 per cent w/v solution of *digoxin impurity C IPRS* in *methanol*.

Reference solution (d). A 0.05 per cent w/v solution of *digoxin impurity H IPRS* in *methanol*. To 1.0 ml of the solution, add 1.0 ml of the test solution and dilute to 20.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile* and 90 volumes of *water*,
  B. a mixture of 10 volumes of *water* and 90 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	78	22
5	78	22
15	30	70
18	78	22

Name	Relative retention time
Digoxin impurity C[1]	0.3
Digoxin impurity E[2]	0.5
Digoxin impurity F[3]	0.6
Digoxin impurity G[4]	0.8
Digoxin (Retention time: about 4.3 minutes)	1.0
Digoxin impurity L[5]	1.4
Digoxin impurity K[6]	1.6
Digoxin impurity B[7]	2.2
Digoxin impurity A[8]	2.6
Digoxin impurity H[9]	---

[1]digoxigenin,
[2]diginatin,

[3]digoxigenin bisdigitoxoside,

[4]neodigoxin,

[5]unknown strucuture,

[6]digoxigenin tetrakisdigitoxoside,

[7]gitoxin,

[8]digitoxin,

[9]lanatoside C.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to digoxin impurity H and digoxin is not less than 1.5.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to digoxin impurity F is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent), the area of peak corresponding to digoxin impurity C is not more than 5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of peak corresponding to digoxin impurities E and K is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of peak corresponding to digoxin impurity G is not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent), the area of peak corresponding to digoxin impurities A and B is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of peak corresponding to digoxin impurity L is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks other than digoxin impurities A, B, C, E, F, G, K and L is not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.7 per cent) and the sum of areas of all the secondary peaks is not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on the residue obtained from the test for Loss on drying.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying over *phosphorus pentoxide* at a pressure not exceeding 2.7 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 200.0 ml of *ethanol (95 per cent)*.

Reference solution (a). A 0.025 per cent w/v solution of *digoxin IPRS* in *ethanol (95 per cent)*.

Reference solution (b). A 0.004 per cent w/v solution each of *digoxin IPRS* and *digoxin impurity C IPRS* in *ethanol (95 per cent)*.

Chromatographic system

- a stainless steel column 25 cm x 4.2 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 37 volumes of *water* and 13 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume: 10 µl.

Inject reference solution (b). The resolution between the peaks due to digoxin and digoxin impurity C is not less than 4.0.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 1200 theoretical plates and tailing factor for the principal peak is not more than 2.0 and the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{41}H_{64}O_{14}$.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Digoxin Injection

Digoxin Injection is a sterile solution of Digoxin in Water for Injections and Ethanol or other suitable solvents.

Digoxin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of digoxin, $C_{41}H_{64}O_{14}$.

Usual strength. 250 µg per ml.

Identification

Evaporate 2 ml to dryness, dissolve the residue in 1 ml of *glacial acetic acid* containing 0.01 per cent w/v of *ferric chloride* and cautiously add 1 ml of *sulphuric acid* without mixing; a brown ring develops at the interface which gradually becomes blue and finally the upper layer acquires a blue colour.

Tests

pH (2.4.24). 6.7 to 7.3.

Bacterial endotoxins (2.2.3). Not more than 200.0 Endotoxin Units per mg of digoxin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Transfer 20.0 ml, measured, to a separating funnel containing 10 ml of *water*. Make alkaline with *5 M ammonia* and extract with four quantities, each of 25 ml, of *chloroform*. Wash each extract with the same 10 ml of *water*. Evaporate the combined extracts to dryness on a water-bath, dissolve the residue in 5.0 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and add 20.0 ml of *glacial acetic acid* (solution A). To 5.0 ml of a 0.2 per cent w/v solution of *digoxin IPRS* in *glacial acetic acid* add 10.0 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and sufficient *glacial acetic acid* to produce 50.0 ml (solution B). Dilute 5.0 ml of solution A to 25.0 ml with *digoxin reagent*, mix, allow to stand for 1 hour and measure the absorbance of the resulting solution at 590 nm, using *water* as the blank (2.4.7). Calculate the content of $C_{41}H_{64}O_{14}$ from the absorbance obtained by treating 5.0 ml of solution B at the same time and in the same manner.

Storage. Store protected from light in single dose containers.

Digoxin Paediatric Solution

Paediatric Digoxin Elixir

Digoxin Paediatric Solution is a solution of Digoxin in a suitable flavoured vehicle.

Digoxin Paediatric Solution contains not less than 90.0 per cent and not more than 110.0 per cent w/v of the stated amount of digoxin, $C_{41}H_{64}O_{14}$.

Usual strength. 50 µg per ml.

Identification

Digoxin Paediatric Solution should not be diluted before use and should be measured with a pipette.

Extract a quantity of the solution containing 250 µg of Digoxin with four quantities, each of 20 ml, of *chloroform*, washing each extract with the same 10 ml of *water*, evaporate the combined extracts to dryness and dissolve the residue in 1 ml of *glacial acetic acid* containing 0.01 per cent w/v of *ferric chloride*. Add cautiously 1 ml of *sulphuric acid* without mixing; a brown ring develops at the interface which gradually becomes blue and finally the upper layer acquires a blue colour.

Tests

pH (2.4.24). 6.8 to 7.2.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Extract a measured volume containing about 5 mg of Digoxin with four quantities, each of 25 ml, of *chloroform*, washing each extract with the same 5 ml of *water*, and evaporate the combined extracts to dryness. To the residue add 3 ml of *ethanol* and carefully evaporate to dryness on a water-bath with the aid of a gentle current of air. Repeat the evaporation using a further 3 ml of *ethanol* and cool. Dissolve the residue in 5.0 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol*, add 20.0 ml of *glacial acetic acid* and filter if necessary. Dilute 5.0 ml of the filtrate to 25.0 ml with digoxin reagent, allow to stand for 1 hour and measure the absorbance of the resulting solution at the maximum at 590 nm (2.4.7). Calculate the content of $C_{41}H_{64}O_{14}$ from the absorbance obtained by carrying out the operation described above at the same time but using a solution prepared by mixing 5.0 ml of a 0.2 per cent w/v solution of *digoxin IPRS* in *glacial acetic acid* with 10.0 ml of a mixture of 65 volumes of *chloroform* and 35 volumes of *methanol* and adding sufficient *glacial acetic acid* to produce 50.0 ml beginning at the words "Dilute 5.0 ml of the filtrate....." and using *water* as the blank.

Storage. Store protected from light at a temperature not exceeding 30°.

Digoxin Tablets

Digoxin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of digoxin, $C_{41}H_{64}O_{14}$.

Usual strengths. 62.5 µg; 125 µg; 250 µg.

Identification

To a quantity of the powdered tablets containing 250 µg of Digoxin add 1 ml of *glacial acetic acid* containing 0.01 per cent w/v of *ferric chloride*, shake for a few minutes, filter through sintered-glass and add cautiously 1 ml of *sulphuric acid* to the filtrate without mixing; a brown ring free from red colour is produced at the interface which gradually becomes blue and finally the upper layer acquires an indigo colour.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 600 ml of freshly distilled *water*,
Speed and time. 120 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first 1 ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of *L-ascorbic acid* in *methanol* and 0.2 ml of a 0.009M solution of hydrogen peroxide (prepared by diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with *0.02 M potassium*

permanganate), mix and dilute to volume with *hydrochloric acid*. After exactly 2 hours measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 360 nm and an emission wavelength of 490 nm and setting the spectrophotofluorimeter to zero with *water* and to 100 with a solution prepared at the same time as the test solution in the following manner. Dilute 2.5 ml of a 0.1 per cent w/v solution of *digoxin IPRS* in *ethanol (80 per cent)* to 100.0 ml with *water*, dilute the resulting solution further with *water* to produce a solution containing in 1 ml an amount of digoxin equal to one-hundredth of the strength of the tablets under examination, transfer 1.0 ml of the solution to a 10-ml volumetric flask and carry out the operation described above, beginning at the words "add 3.0 ml....".

Q. Not less than 75 per cent of the stated amount of digoxin, $C_{41}H_{64}O_{14}$.

Uniformity of content. Complies with the test stated under Tablets.

Test solution. For tablets containing 250 µg of Digoxin, place 1 tablet with 10 ml of *water* at 37°, agitate to disintegrate, add 56 ml of *ethanol (95 per cent)*, shake for 60 minutes and add sufficient *ethanol (80 per cent)* to produce 100.0 ml.

For tablets containing 125 µg and 62.5 µg of Digoxin, repeat the above procedure by using proportionately smaller quantities of *water*, *ethanol (95 per cent)* and *ethanol (80 per cent)*.

Filter through a suitable membrane filter disc having an average pore diameter not greater than 0.8 µm, rejecting the first few ml of the filtrate. Transfer 1.0 ml to a 10-ml volumetric flask, add 3.0 ml of a 0.1 per cent w/v solution of *L-ascorbic acid* in *methanol* and 0.2 ml of a 0.009M solution of hydrogen peroxide [prepared by diluting *hydrogen peroxide solution (100 vol)* that has been standardised by titration with *0.02 M potassium permanganate*], mix and dilute to volume with *hydrochloric acid*. After exactly 2 hours measure the fluorescence of the solution (2.4.5), using an excitation wavelength of about 360 nm and an emission wavelength of about 490 nm and setting the spectrophotofluorimeter to zero with *water*. Calculate the content of digoxin, $C_{41}H_{64}O_{14}$, from the fluorescence obtained by carrying out the operation described above at the same time using a 0.00025 per cent w/v solution of *digoxin IPRS* in *ethanol (80 per cent)* and beginning at the words "Transfer 1.0 ml to a 10-ml volumetric flask...."

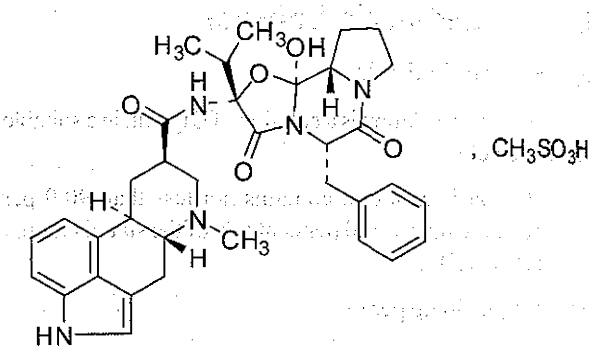
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 1.25 mg of Digoxin, add 3.0 ml of *water*, swirl to disperse the powder and allow to stand for 10 minutes, swirling occasionally. Add 25.0 ml of *glacial acetic acid*, shake for 1 hour and filter, discarding the first few ml of the filtrate. To 4.0 ml of the filtrate add 1.0 ml of *dimethyl sulphoxide*, *dilute* to 25.0 ml with *xanthydrol reagent*, mix well and allow to stand in the dark for 4½ hours (solution A). At the same time prepare two further solutions in the same manner but using for solution B 4.0 ml of *digoxin standard solution* and for solution C 4.0 ml of a mixture of 25 volumes of *glacial acetic acid* and 3 volumes of *water* and beginning at the words "add 1.0 ml of *dimethyl sulphoxide*.....". Measure the absorbances of solutions A and B at the maximum at 545 nm (2.4.7), using solution C as the blank. Calculate the content of $C_{41}H_{64}O_{14}$ from the absorbances obtained.

Storage. Store protected from light.

Dihydroergocristine Mesylate

Dihydroergocristine Mesilate



$C_{36}H_{45}N_5O_8S$ Mol. Wt. 708

Dihydroergocristine Mesylate is (5′α)-12′-hydroxy-2′-(1-methylethyl)-5′-(phenylmethyl)dihyroergotaman-3′,6′,18-trione methanesulphonate.

Dihydroergocristine Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{36}H_{45}N_5O_8S$, calculated on the dried basis.

Category. Antimigraine.

Production

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

Description. A white or almost white, fine crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dihydroergocristine mesylate IPRS* or with the reference spectrum of dihydroergocristine mesylate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

Mobile phase. A mixture of 2 volumes of *ammonia*, 15 volumes of *dimethylformamide* and 85 volumes of *ether*.

Test solution. Dissolve 0.1 g of the substance under examination in 5 ml of the same solvent mixture.

Reference solution. A 2.0 per cent w/v solution of *dihydroergocristine mesylate IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 8 cm, protected from light. Dry the plate in current of cold air for 5 minutes. Spray with *dimethylaminobenzaldehyde solution* and dry in a current of hot air for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

Mobile phase. A mixture of 5 volumes of *water*, 10 volumes of *ammonia*, 20 volumes of *butanol* and 65 volumes of *acetone*.

Test solution. Dissolve 0.2 g of the substance under examination in 5 ml of the solvent mixture.

Reference solution. A 0.4 per cent w/v solution of *methanesulphonic acid* in the solvent mixture.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm, protected from light. Dry the plate in current of cold air for not more than 1 minute. Spray with a 0.1 per cent w/v solution of *bromocresol purple* in *methanol*, adjusting the colour to violet-red with one drop of *dilute ammonia solution* and dry the plate in a current of hot air at 100°. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 2.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BS7 (2.4.1).

pH (2.4.24). 4.0 to 5.0, determined in a 0.5 per cent w/v solution in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). −43.0° to −37.0°, determined in a 1.0 per cent w/v solution in *anhydrous pyridine*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Carry out the test protected from light.

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of 0.1 per cent w/v solution of *orthophosphoric acid*.

Test solution. Dissolve 75 mg of the substance under examination in solvent mixture and dilute to 50.0 ml with *water*.

Reference solution. Dissolve 20 mg of *codergocrine mesilate IPRS* in solvent mixture and dilute to 50.0 ml with *water*. Dilute 6.0 ml of the solution to 50.0 ml with a mixture of 20 volumes of *acetonitrile*, 20 volumes of 0.1 per cent w/v solution of *orthophosphoric acid* and 60 volumes of *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 10 volumes of *acetonitrile*, 90 volumes of *water* and 1 volume of *triethylamine*,
  B. a mixture of 10 volumes of *water*, 90 volumes of *acetonitrile* and 1 volume of *triethylamine*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
5	75	25
20	25	75
22	75	25
30	75	25

Inject the reference solution. The relative retention time with reference to dihydroergocristine for dihydroergocornine (dihydroergocristine impurity F) is about 0.8; for α-dihydroergocryptine (dihydroergocristine impurity H) is about 0.9; for α-dihydroergocryptine or epicriptine (dihydroergocristine impurity I) is about 1.02. In the chromatogram shows 4 peaks, the resolution between the peaks corresponding to dihydroergocristine and dihydroergocristine impurity I is not less than 1.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent), the sum of the areas of all the secondary peaks

is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (0.1 per cent).

Loss on drying (2.4.19). Not more than 3 per cent, determined on 0.5 g by drying under vacuum, in an oven at 80°.

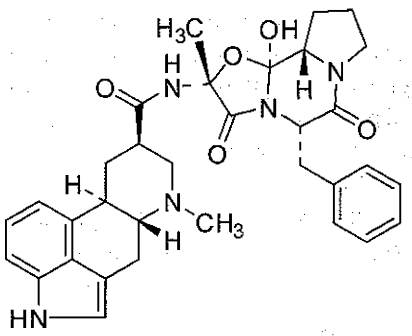
Assay. Dissolve 0.3 g in 60 ml of *pyridine*. Pass a stream of nitrogen over the surface of the solution. Titrate with *0.1 M tetrabutylammonium hydroxide* to the second point of inflexion, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03539 g of $C_{36}H_{45}N_5O_8S$.

Storage. Store protected from light.

Dihydroergotamine Mesylate

Dihydroergotamine Mesilate



, CH_3SO_3H

$C_{33}H_{37}N_5O_5,CH_4SO_3$ Mol. Wt. 680.0

Dihydroergotamine Mesylate is (5′α)-12′-hydroxy-2′-methyl-5′-(phenylmethyl)dihyroergotaman-3′,6′,18-trione methanesulphonate.

Dihydroergotamine Mesylate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{33}H_{37}N_5O_5,CH_4SO_3$, calculated on the dried basis.

Category. Antimigraine.

Production

The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

Description. A white or almost white, crystalline powder or crystals.

Identification

Tests A and C may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dihydroergotamine mesylate IPRS* or with the reference spectrum of dihydroergotamine mesylate.

B. When examined in the range 250 nm to 350 nm (2.4.7), a 0.005 per cent w/v solution in *methanol*, shows absorption maxima, at about 281 nm and 291 nm and a shoulder at 275 nm; specific absorbance at about 281 nm is 95 to 105.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to the principal spot in the chromatogram obtained with reference solution (a).

D. To 0.1 g of the substance under examination, add 5 ml of *dilute hydrochloric acid*, shake for about 5 minutes and filter. Add 1 ml of *barium chloride solution*. The filtrate remains clear. Mix 0.1 g of the substance under examination with 0.4 g of powdered *sodium hydroxide*, heat to fusion and continue to heat for 1 minute. Cool, add 5 ml of *water*, boil and filter. Acidify the filtrate with *hydrochloric acid* and filter again. The filtrate gives reaction (A) of sulphates (2.3.1).

Tests

Appearance of solution. Dissolve 0.1 g in a mixture of 0.1 ml of a 7.0 per cent w/v solution of *methanesulphonic acid* and 50 ml of *water*. The solution is clear (2.4.1) and not more intensely coloured than reference solution YS7 or BYS7 (2.4.1).

pH (2.4.24). 4.4 to 5.4, determined on 0.1 per cent w/v solution in *carbon dioxide- free water*.

Specific optical rotation (2.4.22). −47.0° to −42.0°, determined in a 1.0 per cent w/v solution in *anhydrous pyridine*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

NOTE—Prepare the solutions immediately before use.

Solvent mixture. 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

Mobile phase. A mixture of 1 volume of *ammonia*, 6 volumes of *methanol*, 50 volumes of *ethyl acetate* and 50 volumes of *dichloromethane*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 5 ml of the solvent mixture.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with the solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of *dihydroergotamine mesilate IPRS* in the solvent mixture.

Reference solution (b). Dilute 2.5 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Reference solution (c). Dilute 2.0 ml of reference solution (b) to 5.0 ml with the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm, protected from light. Dry the plate in current of cold air for not more than 1 minute and repeat the development, allow the freshly prepared mobile phase to rise 15 cm. Spray abundantly with *dimethylaminobenzaldehyde solution* and dry in a current of hot air for about 2 minutes. In the chromatogram obtained with test solution (a), any secondary spot is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and not more than 2 such spots are more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

Loss on drying (2.4.19). Not more than 4.0 per cent, determined on 0.5 g by drying in an oven at 105° at a pressure not exceeding 0.1 kPa for 5 hours.

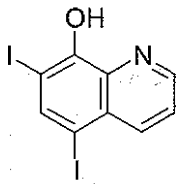
Assay. Dissolve 0.5 g in a mixture of 10 ml of *anhydrous acetic acid* and 70 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid is* equivalent to 0.068 g of $C_{34}H_{41}N_5O_8S$.

Storage. Store protected from light.

Diiodohydroxyquinoline

Iodoquinol



$C_9H_5I_2NO$ Mol. Wt. 396.9

Diiodohydroxyquinoline is 5,7-diiodoquinolin-8-ol.

Diiodohydroxyquinoline contains not less than 97.0 per cent and not more than 100.5 per cent of $C_9H_5I_2NO$, calculated on the dried basis.

Category. Antiamoebic.

Description. A light yellowish to yellowish-brown, microcrystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diiodohydroxyquinoline IPRS* or with the reference spectrum of diiodohydroxyquinoline.

B. Dissolve 10 mg in 100 ml of *dioxan* and dilute 5 ml to 100 ml with *ethanol*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 258 nm; absorbance at about 258 nm, about 0.53.

C. Heat a few crystals with about 1 ml of *sulphuric acid;* violet vapours of iodine are evolved.

Tests

Acidity or alkalinity. Shake 0.5 g with 10 ml of *water* previously neutralised to *phenolphthalein solution*. The solution is colourless and not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Free iodine and iodide. Shake 1.0 g with 20 ml of *water* for 30 seconds, allow to stand for 5 minutes and filter. To 10 ml of the filtrate add 1 ml of *1 M sulphuric acid* and 2 ml of *chloroform* and shake; the chloroform layer does not become violet. To the mixture add 5 ml of *1 M sulphuric acid* and 1 ml of *potassium dichromate solution* and shake for 15 seconds; the colour of the chloroform layer does not become more intense than that produced by diluting 2 ml of a 0.016 per cent w/v solution of *potassium iodide* to 10 ml with *water*, adding 6 ml of *1 M sulphuric acid*, 1 ml of *potassium dichromate solution* and 2 ml of *chloroform* and shaking for 15 seconds.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Add 0.5 ml of *N,O-bis (trimethylsilyl)acetamide* to 0.5 ml of a solution in *pyridine* containing 0.4 per cent w/v of each of *5-chloro-8-hydroxyquinoline, 5,7-dichloro-8-hydroxy-quinoline* and *5-chloro-7-iodo-8-hydroxy-quinoline* and 0.04 per cent w/v of the substance under examination, mix, allow to stand for 15 minutes and add 5 ml of a 0.05 per cent w/v solution of *dibutylphthalate* (internal standard) in *hexane*.

Reference solution (a). Add 0.5 ml of *N,O-bis (trimethylsilyl) acetamide* to a mixture of 0.1 g of the substance under examination and 0.5 ml of *pyridine,* mix, allow to stand for 15 minutes and add 5 ml of *hexane*.

Reference solution (b). Treat a mixture of 0.1 g of the substance under examination and 0.5 ml of *pyridine* as described for the test solution.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of methyl silicone gum,
- temperature:
  column 190°,
  inlet port and detector 240°,
- flame ionisation detector,
- nitrogen as the carrier gas.

In the chromatogram obtained with the test solution the peaks following the solvent peak, in order of emergence, are due to (a) 5-chloro-8-hydroxyquinoline, (b) 5,7-dichloro-8-hydroxyquinoline, (c) the internal standard, (d) 5-chloro-7-iodo-8-hydroxyquinoline and (e) diiodohydroxyquinoline. In the chromatogram obtained with reference solution (b) calculate the content of 5-chloro-8-hydroxy-quinoline, 5,7-dichloro-8-hydroxyquinoline and 5-chloro-7-iodo-8-hydroxyquinoline by reference to the corresponding peaks in the chromatogram obtained with the test solution. The total content of the named impurities and any other impurities does not exceed 4.0 per cent w/w.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Dissolve 0.3 g in 50 ml of *anhydrous pyridine*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03969 g of $C_9H_5I_2NO$.

Storage. Store protected from light.

Diiodohydroxyquinoline Tablets

Iodoquinol Tablets

Diiodohydroxyquinoline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diiodohydroxyquinoline, $C_9H_5I_2NO$.

Usual strengths. 300 mg; 600 mg.

Identification

A. Triturate a quantity of the powdered tablets containing about 50 mg of Diiodohydroxyquinoline with 10 ml of *carbon disulphide*, filter and evaporate the solvent. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diiodohydroxyquinoline IPRS* or with the reference spectrum of diiodohydroxyquinoline.

B. Shake a quantity of the powdered tablets containing about 10 mg of Diiodohydroxyquinoline with 100 ml of *dioxan*, filter and dilute 5 ml of the filtrate to 100 ml with *ethanol*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 258 nm; absorbance at about 258 nm, about 0.53 (2.4.7).

Tests

Soluble iodides. Digest a quantity of the powdered tablets containing 0.1 g of Diiodohydroxyquinoline with 5 ml of *water* for 10 minutes, cool and filter. To the filtrate add 1 ml of *3 M hydrochloric acid*, 0.1 ml of *ferric chloride test solution* and 2 ml of *chloroform*, shake gently and allow to separate; any violet colour in the chloroform is not more intense than that in a blank to which 1 ml of a 0.02 per cent w/v solution of *potassium iodide* has been added.

Disintegration (2.5.1). Not more than 30 minutes.

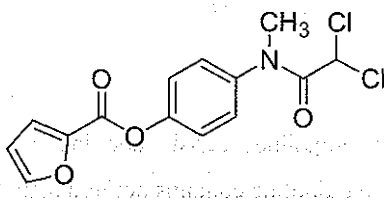
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 12 mg of Diiodohydroxyquinoline and determine by the oxygen-flask method (2.3.34), using a mixture of 10 ml of *water* and 2 ml of *1 M sodium hydroxide* as the absorbing liquid. When the process is complete, add to the flask an excess (5 ml to 10 ml) of *acetic bromine solution* and allow to stand for 2 minutes. Remove the excess of bromine by the addition of *formic acid* (about 0.5 ml to 1 ml). Rinse the sides of the flask with *water* and sweep out any bromine vapour above the liquid with a current of air. Add 1 g of *potassium iodide* and titrate with *0.02 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as the indicator.

1 ml of *0.02 M sodium thiosulphate* is equivalent to 0.0006616 g of $C_9H_5I_2NO$.

Storage. Store protected from light.

Diloxanide Furoate



$C_{14}H_{11}Cl_2NO_4$ Mol. Wt. 328.2

Diloxanide Furoate is 4-(*N*-methyl-2,2-dichloroacetamido) phenyl 2-furoate.

Diloxanide Furoate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{11}Cl_2NO_4$, calculated on the dried basis.

Category. Antiamoebic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diloxanide furoate IPRS* or with the reference spectrum of diloxanide furoate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum only at about 258 nm; absorbance at about 258 nm, about 0.70.

C. On 20 mg determine by the oxygen-flask method (2.3.34), using 10 ml of *1 M sodium hydroxide* as the absorbing liquid. When the process is complete, acidify the liquid with *nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

Tests

Free acidity. Shake 3.0 g with 50 ml of *water*, filter and wash the residue with three quantities, each of 20 ml, of *water*. Titrate the combined filtrate and washings with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator; not more than 1.3 ml is required.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 96 volumes of *dichloromethane* and 4 volumes of *methanol*.

Test solution. Dissolve 0.5 g of the substance under examination in 5 ml of *chloroform*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *chloroform* and mix. Dilute 5.0 ml of the resulting solution to 20.0 ml with *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.3 g in 50 ml of *anhydrous pyridine*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03282 g of $C_{14}H_{11}Cl_2NO_4$.

Storage. Store protected from light.

Diloxanide Tablets

Diloxanide Furoate Tablets

Diloxanide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of diloxanide furoate, $C_{14}H_{11}Cl_2NO_4$.

Usual strength. 500 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.2 g of Diloxanide Furoate with 20 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diloxanide furoate IPRS* or with the reference spectrum of diloxanide furoate.

B. On 20 mg of the residue obtained in test A determine by the oxygen-flask method (2.3.34), using 10 ml of *1 M sodium hydroxide* as the absorbing liquid. When the process is complete, acidify the liquid with *nitric acid* and add *silver nitrate solution*; a white precipitate is produced.

C. The residue obtained in test A melts at 114° to 116° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 96 volumes of *dichloromethane* and 4 volumes of *methanol*.

Test solution. Shake a quantity of the powdered tablets containing 0.5 g of Diloxanide Furoate with 5 ml of *chloroform*, centrifuge and use the supernatant liquid.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *chloroform* and mix. Dilute 5.0 ml of the resulting solution to 20.0 ml with *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

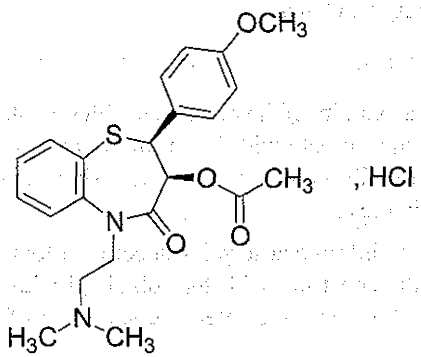
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 40 mg of Diloxanide Furoate, shake with 150 ml of *ethanol (95 per cent)* for 30 minutes, add

sufficient *ethanol (95 per cent)* to produce 200.0 ml, mix and filter. Dilute 10.0 ml of the filtrate to 250.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of $C_{14}H_{11}Cl_2NO_4$ taking 705 as the specific absorbance at 258 nm.

Storage. Store protected from light.

Diltiazem Hydrochloride



$C_{22}H_{26}N_2O_4S,HCl$ Mol. Wt. 451.0

Diltiazem Hydrochloride is (*2S,3S*)-2,3,4,5-tetrahydro-5-(2-dimethylaminoethyl)-2-(4-methoxyphenyl)-4-oxobenzo[*b*] thiazepin-3-yl acetate hydrochloride.

Diltiazem Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{22}H_{26}N_2O_4S,HCl$, calculated on the dried basis.

Category. Antianginal; (calcium-channel blocker).

Description. A white, crystalline powder or small crystals.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diltiazem hydrochloride IPRS* or with the reference spectrum of diltiazem hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (a).

C. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Specific optical rotation (2.4.22). +110.0° to +116.0°, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.12 g of the substance under examination in *methanol* and dilute 100.0 ml with *methanol*.

Reference solution (a). A 0.12 per cent w/v solution of *diltiazem hydrochloride IPRS* in *methanol*.

Reference solution (b). A solution containing 0.0012 per cent w/v each of *diltiazem hydrochloride IPRS* and *desacetyl diltiazem hydrochloride IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution containing 0.116 per cent w/v of *d-10-camphorsulphonic acid* in *0.1 M sodium acetate*, adjusted to pH 6.2 with *0.1 M sodium hydroxide*, 25 volumes of *acetonitrile* and 25 volumes of *methanol*,
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

The relative retention time with reference to diltiazem for desacetyl diltiazem is about 0.65.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to desacetyl diltiazem and diltiazem is not less than 3, the column efficiency is not less than 1200 theoretical plates and the relative standard deviation for replicate injections is not more than 10.0 per cent for diltiazem peak.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to desacetyl diltiazem is not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals. Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances using the following modifications.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{26}N_2O_4S,HCl$.

Storage. Store protected from light.

Diltiazem Injection

Diltiazem Hydrochloride Injection

Diltiazem Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diltiazem hydrochloride, $C_{22}H_{26}N_2O_4S$, HCl.

Usual strength. 25 mg per 5 ml; 50 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.7 to 4.2.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing 0.12 g of Diltiazem hydrochloride to 100.0 ml with *methanol.*

Reference solution. A solution containing 0.0012 per cent w/v each of *diltiazem hydrochloride IPRS* and *desacetyl diltiazem hydrochloride IPRS* in *methanol.*

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution containing 0.116 per cent w/v of *d-10-camphorsulphonic acid* in *0.1 M sodium acetate*, adjusted to pH 6.2 with *0.1 M sodium hydroxide*, 25 volumes of *acetonitrile* and 25 volumes of *methanol,*
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

The relative retention time with reference to diltiazem for desacetyl diltiazem is about 0.65.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to desacetyl diltiazem and diltiazem is not less than 3, the column efficiency is not less than 1200 theoretical plates and the relative standard deviation for replicate injections is not more than 10.0 per cent for diltiazem peak.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to desacetyl diltiazem is not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (1.5 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 1.4 Endotoxin Units per mg of diltiazem hydrochloride.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing 50 mg of Diltiazem Hydrochloride to 50.0 ml with *methanol.* Dilute 5.0 ml of the solution to 50.0 ml with *methanol.*

Reference solution. A 0.01 per cent w/v solution of *diltiazem hydrochloride IPRS* in *methanol.*

Chromatographic system

- a stainless steel column 30 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a 0.005 per cent w/v solution of *heptane sulphonic acid* in a mixture of 35 volumes of *acetonitrile*, 5 volumes of *methanol* and 60 volumes of 0.46 per cent w/v of *ammonium acetate*, adjusted to pH 6.0 with *glacial acetic acid,*
- spectrophotometer set at 240 nm,
- flow rate: 1.6 ml per minute,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{26}N_2O_4S$, HCl in the injection.

Storage. Store under refrigeration between 2° to 8°. Do not allow to freeze.

Labelling. Label as for direct intravenous bolus injection and continuous intravenous infusion. Single use container, discard unused portion.

Diltiazem Tablets

Diltiazem Hydrochloride Tablets

Diltiazem Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diltiazem hydrochloride, $C_{22}H_{26}N_2O_4S$, HCl. They may be Modified-release Tablets.

Usual strengths. 30 mg; 60 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to diltiazem hydrochloride in the chromatogram obtained with the reference solution (a).

Tests

Dissolution (2.5.2).

A. *For Modified-release Tablets*

Apparatus No. 2 (Paddle),

Medium. 900 ml of freshly distilled *water,*

Speed and time. 100 rpm and 30 minutes and 3 hours.

Withdraw a suitable volume of the medium after 30 minutes and 3 hours. Filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute a suitable volume of the filtrate with the same solvent and measure the absorbance of the resulting solution at the maximum at 240 nm (2.4.7). Calculate the content of $C_{22}H_{26}N_2O_4S$,HCl from the absorbance obtained from a solution of known concentration of *diltiazem hydrochloride IPRS*.

Use the following acceptance criteria for the 30-minute time interval. At S_1: no unit is more than Q; at S_2, the average value is equal to or less than Q, and no unit is greater than Q + 10 per cent; at S_3, the average value is equal to or less than Q, not more than 2 units are more than Q + 10 per cent and no unit is more than Q + 25 per cent. Use the acceptance criteria in Acceptance Table 1(2.5.2) for the 3-hour time interval.

Q. Not more than 60 per cent of the stated amount of $C_{22}H_{26}N_2O_4S$,HCl is dissolved in 30 minutes and not less than 80 per cent is dissolved in 3 hours.

B. *For Conventional-release Tablets*

Apparatus No. 2 (Paddle),

Medium. 900 ml of freshly distilled *water,*

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium. Filter promptly, rejecting the first few ml of the filtrate. Dilute a suitable volume of the filtrate with the same solvent and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{22}H_{26}N_2O_4S$,HCl from the absorbance obtained from a solution of known concentration of *diltiazem hydrochloride IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{26}N_2O_4S$,HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.12 g of Diltiazem Hydrochloride in *methanol* with the aid of ultrasound for 60 minutes and dilute to 100.0 ml with *methanol*. Centrifuge the solution for 20 minutes and use the supernatant.

Reference solution. A solution containing 0.0012 per cent w/v each of *diltiazem hydrochloride IPRS* and *desacetyl diltiazem hydrochloride IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution containing 0.116 per cent w/v of *d-10-camphorsulphonic acid* in *0.1 M sodium acetate*, adjusted to pH 6.2 with *0.1 M sodium hydroxide*, 25 volumes of *acetonitrile* and 25 volumes of *methanol,*
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

The relative retention time with reference to diltiazem for desacetyl diltiazem is about 0.65.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to desacetyl diltiazem and diltiazem is not less than 3, the column efficiency is not less than 1200 theoretical plates and the relative standard deviation for replicate injections is not more than 10.0 per cent for diltiazem peak.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to desacetyl diltiazem is not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (1.5 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.6 g of Diltiazem Hydrochloride, add 200 ml of *methanol*, mix with the aid of ultrasound for 1 hour, cool and dilute to 500.0 ml with *methanol*; centrifuge 25 ml at 3500 rpm for 15 minutes and use the clear, supernatant liquid.

Reference solution (a). A solution containing 0.0012 per cent w/v each of *diltiazem hydrochloride IPRS* and *desacetyl diltiazem hydrochloride IPRS* in *methanol.*

Reference solution (b). Dissolve 60 mg of *diltiazem hydrochloride IPRS* in 50 ml of *methanol.*

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution containing 0.116 per cent w/v of d-*10-camphorsulphonic acid* in *0.1 M sodium acetate*, adjusted to pH 6.2 with *0.1 M sodium hydroxide*, 25 volumes of *acetonitrile* and 25 volumes of *methanol,* filtered and degassed,
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Inject reference solution (a) and measure the peak responses of all the peaks. The relative retention times for desacetyl diltiazem and diltiazem are about 0.65 and 1.0 respectively. The resolution between desacetyl diltiazem and diltiazem is not less than 3, and the theoretical plates for the diltiazem peak is not less than 1200.

Inject reference solution (b).The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

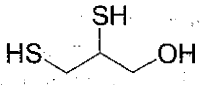
Inject reference solution (b) and the test solution.

Calculate the content of $C_{22}H_{26}N_2O_4S,HCl$ in the tablets..

Storage. Store protected from light.

Dimercaprol

B.A.L.



$C_3H_8OS_2$ Mol. Wt. 124.2

Dimercaprol is (*RS*)-2,3-dimercaptopropanol.

Dimercaprol contains not less than 98.5 per cent w/w and not more than 101.5 per cent w/w of $C_3H_8OS_2$.

Category. Antidote in heavy metal poisoning; metal complexing agent.

Description. A clear, colourless or slightly yellow liquid.

Identification

A. Dissolve 0.1 ml in 4 ml of *water* and to 2 ml of the solution add *lead acetate solution*; a yellow precipitate is obtained.

B. To 2 ml of the solution prepared for test A add 1 ml of *0.05 M iodine*; the colour of iodine is immediately discharged.

C. In a ground-glass-stoppered tube suspend 0.6 g of *sodium bismuthate*, previously heated to 200° for 2 hours, in a mixture of 6 ml of *water* and 2.8 ml of a 10 per cent w/w solution of *phosphoric acid*. Add 0.2 ml of the substance under examination, mix and allow to stand for 10 minutes shaking frequently. To 1 ml of the supernatant liquid add 5 ml of a 0.4 per cent w/v solution of *chromotropic acid sodium salt* in *sulphuric acid*, mix and heat for 15 minutes in a water-bath; a violet-red colour is produced.

Tests

Appearance of solution. The substance under examination is clear (2.4.1), and not more intensely coloured than reference solution BS6 or BYS6 (2.4.1).

pH (2.4.24). 5.0 to 6.5, determined in a saturated solution.

Refractive index (2.4.27). 1.568 to 1.574, determined at 20°.

Weight per ml (2.4.29). 1.238 g to 1.240 g.

Iron (2.3.14). Ignite 2.0 g with 1 g of *anhydrous sodium carbonate*, cool, dissolve the residue in 15 ml of *dilute hydrochloric acid* and dilute to 45 ml with *water*; the resulting solution complies with the limit test for iron (20 ppm).

Halides. To 2.0 g add 25 ml of *0.5 M ethanolic potassium hydroxide* and heat under a reflux condenser for 2 hours. Remove the ethanol by evaporation in a current of warm air, add 20 ml of *water* and cool. Add a mixture of 10 ml of *strong hydrogen peroxide solution* and 40 ml of *water*. Boil gently for 10 minutes; cool and filter rapidly. Add 10 ml of *dilute nitric acid* and 5 ml of *0.1 M silver nitrate* and titrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator. Repeat the operation without the substance under examination. The difference in the volumes of *0.1 M ammonium thiocyanate* used in the two titrations is not more than 1.0 ml.

Assay. Dissolve 0.1 g in 40 ml of *methanol* and add 20 ml of *0.1 M hydrochloric acid* and 50.0 ml of *0.05 M iodine*. Allow to stand for 10 minutes and titrate with *0.1 M sodium thiosulphate*. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine required.

1 ml of *0.05 M iodine* is equivalent to 0.00621 g of $C_3H_8OS_2$.

Storage. Store protected from light in well-filled containers in a refrigerator (2° to 8°).

Dimercaprol Injection

B.A.L. Injection

Dimercaprol injection is a sterile solution of Dimercaprol in a mixture of Benzyl Benzoate and Arachis Oil.

Dimercaprol Injection contains not less than 90.0 per cent and more than 110.0 per cent of the stated amount of dimercaprol, $C_3H_8OS_2$.

Usual strength. 50 mg per ml.

Description. A clear, yellow, viscous solution, having a pungent.

Tests

Acidity. Shake with an equal volume of *water* for 2 minutes and set aside for separation; pH of the aqueous layer after filtration through a neutral filter is 4.5 to 6.5 (2.4.24).

Refractive index (2.4.27). 1.481 to 1.486, determined at 20°.

Weight per ml (2.4.29). About 0.95 g.

Bacterial endotoxins (2.2.3). Not more than 1.0 Endotoxin Unit per mg of dimercaprol.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Weigh 1.0 g, add 20 ml of *0.1 M hydrochloric acid* and titrate with *0.05 M iodine*.

1 ml of *0.05 M iodine* is equivalent to 0.00621 g of $C_3H_8OS_2$.

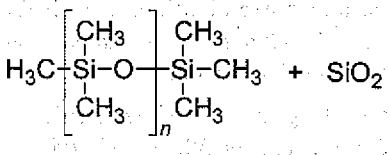
Determine the weight per ml of the injection (2.4.29), and calculate the content of $C_3H_8OS_2$.

Storage. Store protected from light.

Labelling. The label states (1) the nature of the solvent; (2) that the injection is meant for intramuscular use only.

Activated Dimethicone

Simethicone; Activated Polydimethylsiloxane



$(CH_3)_3Si[OSi(CH_3)_2]_nCH_3+SiO_2$

Activated Dimethicone is a mixture of fully methylated linear siloxane polymers containing repeating $-(CH_3)_2SiO-$ units stabilised with trimethylsiloxy, $(CH_3)_3SiO-$, end-blocking units and finely divided silicon dioxide.

Activated Dimethicone contains not less than 90.0 per cent and not more than 99.0 per cent of polydimethylsiloxane, $[-(CH_3)_2SiO-]_n$ and not less than 4.0 per cent and not more than 7.0 per cent of silicon dioxide, SiO_2.

Category. Defoaming agent.

Description. A translucent, grey viscous liquid.

Identification

A. To 50 mg add 25 ml of *toluene* and swirl to disperse. Add 50 ml of *dilute hydrochloric acid* and shake for 5 minutes. Transfer to a separating funnel and remove about 5 ml of the upper layer to a stoppered tube containing 0.5 g of *anhydrous sodium sulphate*. Shake vigorously and centrifuge the mixture until a clear supernatant liquid is obtained. The resulting liquid complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dimethicone IPRS*. Ignore the region of the spectrum from 850 to 750 cm^{-1} since slight differences may be observed depending on the degree of polymerisation.

B. Heat 0.5 g in a test-tube over a small flame until white fumes are evolved. Invert the test-tube over a second tube containing 1 ml of a 0.1 per cent w/v solution of *chromotropic acid sodium salt* in *sulphuric acid* so that the fumes reach the solution. Shake the second tube for about 10 seconds and heat on a water-bath for 5 minutes; the solution is violet.

C. To 50 mg in a platinum crucible add 0.15 ml of *sulphuric acid* and ignite until a white residue is obtained; the residue gives the reaction of silicates (2.3.1).

Tests

Acidity. To 2.0 g add 25 ml of a mixture of equal volumes of *ethanol* and *ether* previously neutralised to 0.2 ml of *bromothymol blue solution* and shake; not more than 3.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to blue.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Defoaming activity. Weigh 0.2 g, transfer to a 100-ml bottle, add 50 ml of *2-methylpropanol* and shake vigorously, warming, if necessary, to effect solution. Add dropwise, 0.5 ml of the solution to a clean, unused, cylindrical 250-ml glass jar, fitted with a 50-mm cap, containing 100 ml of a 1.0 per cent w/v solution of *octoxinol*. Cap the jar and clamp it in an upright position in a wrist-action shaker capable of moving the jar through a radius of 13.3 ± 0.4 cm (measured from the centre of the shaft to the centre of the jar) and an arc of 10 degrees at a frequency of 300 ± 30 strokes per minute. Shake for 10 seconds and record the time required in seconds for the foam to collapse. The time for foam collapse is determined at the instant the first portion of foam-free liquid surface appears, measured from the end of the shaking period. The defoaming activity time is not more than 15 seconds.

Assay. *For polydimethylsiloxane* — Weigh 50 mg, transfer to a narrow-mouthed glass bottle and add 25 ml of *toluene*. Swirl to disperse, add 50 ml of *dilute hydrochloric acid*, close the bottle securely with a cap having an inert liner and shake for exactly 5 minutes. Transfer the mixture to a 125-ml separating funnel and remove about 5 ml of the upper layer to a stoppered test-tube containing 0.5 g of *anhydrous sodium sulphate*. Close the test-tube, agitate vigorously and centrifuge the mixture until a clear supernatant liquid is obtained. Prepare a blank by mixing 10 ml of *toluene* with 0.5 g of *anhydrous sodium sulphate* and centrifuging to obtain a clear supernatant liquid. Determine the absorbance of a 0.5-mm layer of the solution at the maximum at about 7.9 µm in a suitable infra-red spectrophotometer (2.4.6), using the blank to set the instrument. Calculate the content of $[-(CH_3)_2SiO-]_n$ from the absorbance obtained by repeating the Assay on a 0.2 per cent w/v solution of *dimethicone IPRS* in place of the substance under examination.

For silicon dioxide — Mix thoroughly and Weigh 1.0 g; transfer to a tared, sintered-glass filtering crucible (porosity No. 4) and pass through the filter, with suction, 200 ml of *toluene*, added with stirring in small portions, followed by similar washing of the material on the filter with 200 ml of *toluene*, and discard the filtrates. Place the filtering crucible in a muffle furnace at room temperature, raise the temperature of the furnace to 550°. Heat at 550° ± 25° for 2 hours. Cool the filtering crucible with its contents in a desiccator, weigh and calculate the content of silicon dioxide, SiO_2, in the sample taken.

Dimethicone

Dimethicone is α-(Trimethylsilyl)-ω-methylpoly[oxy (dimethylsilylene).

Dimethicone is a mixture of fully methylated linear siloxane polymers containing repeating units of the formula; $[-(CH_3)_2SiO-]_n$ stabilized with trimethylsiloxy end-blocking units of the formula; $[(CH_3)_3SiO-]$ where in *n* has an average value such that the corresponding nominal viscosity is in a discrete range between 20 and 30,000 centistokes.

Dimethicone contains not less than 97.0 per cent and not more than 103.0 per cent of polydimethylsiloxane, $([-(CH_3)_2SiO-]_n)$.

Description. A Clear, colourless liquid of various viscosities.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *polydimethylsiloxane IPRS* or with the reference spectrum of polydimethylsiloxane.

Tests

Specific gravity (2.4.29). Within the limits specified in the accompanying table.

Viscosity (2.4.28). Determine the viscosity of Dimethicone having a nominal viscosity of less than 1000 centistokes at 25° ± 0.1°, using a capillary viscometer. Determine the viscosity of Dimethicone having a nominal viscosity of 1000 centistokes or more at 25° ± 0.1°, using a rotational viscometer. The viscosity is within the limits specified in the accompanying table.

Refractive Index (2.4.27). Within the limits specified in the accompanying table, determined at 25°.

Acidity. Dissolve 15.0 g in a mixture of 15 ml of *toluene* and 15 ml of *butyl alcohol*, previously neutralized to *bromophenol blue*, and titrate with *0.050 M alcoholic potassium hydroxide* to a bromophenol blue endpoint. Not more than 0.1 ml is required.

Loss on heating. Preheat an open aluminium vessel (60 mm in diameter and 10 mm high) at 150° for 30 minutes, and allow to cool to room temperature and weigh. Transfer to it about 1 g of Dimethicone, weighed, heat at 150° in a circulating air oven for 2 hours, and allow to come to room temperature in a desiccator before weighing: it loses not more than the maximum percentage of its weight specified in the accompanying table.

Heavy metals (2.3.13). Mix 1.0 g of Dimethicone with 20 ml of *chloroform*. Add 1.0 ml of a freshly prepared 0.002 per cent w/v solution of *dithizone* in *chloroform*, 0.5 ml of *water*, and 0.5 ml of a mixture of 1 ml of *ammonia* and 9 ml of a 0.2 per cent solution of *hydroxylamine hydrochloride*. Prepare reference solution by adding 1.0 ml of a freshly prepared 0.002 per cent w/v solution of dithizone in *chloroform* to 20 ml of *chloroform*; add 0.5 ml of *lead standard solution (10 ppm pb)* and 0.5 ml of a mixture of 1 ml of *ammonia* and 9 ml of a 0.2 per cent solution of *hydroxylamine hydrochloride*. Immediately shake both solutions vigorously for 1 minute. Any red color in the test solution is not more intense than that in the reference solution (5 µg per g).

Assay. Determine by infra red spectrophotometery (2.4.6), with a resolution of 4 cm^{-1} and fitted with an accessory for attenuated total reflectance and a germanium sample trough (45° or 60°). Fill the trough of the accessory with Dimethicone, and record the spectrum between 4000 cm^{-1} and 700 cm^{-1}. Clean the trough, fill it with *polydimethylsiloxane IPRS* and record the spectrum as above. Clean the trough, and record the spectrum as above to obtain a background spectrum. Examine the spectra in the range between 1300 cm^{-1} and 1200 cm^{-1}, and calculate the absorbance of the peak in each spectrum at about 1259 cm^{-1}.

Calculate the content of $[-(CH_3)_2SiO-]_n$ in the Dimethicone taken by the formula:

$$100(A_U / A_S)(D_S / D_U)$$

in which A_U is the absorbance of the Dimethicone; A_S is the absorbance of *polydimethylsiloxane IPRS*; and D_S and D_U are the specific gravities of *polydimethylsiloxane IPRS* and Dimethicone, respectively.

Nominal Viscosity (centistokes)	Viscosity (centistokes)		Specific gravity		Refractive index		Loss on heating
	Min.	Max.	Min.	Max.	Min.	Max.	Max.
20	18	22	0.946	0.954	1.3980	1.4020	20.0
50	47.5	52.5	0.955	0.965	1.4005	1.4045	2.0
100	95	105	0.962	0.970	1.4005	1.4045	0.3
200	190	220	0.964	0.972	1.4013	1.4053	0.3
350	332.5	367.5	0.965	0.973	1.4013	1.4053	0.3
500	475	525	0.967	0.975	1.4013	1.4053	0.3
1000	950	1050	0.967	0.975	1.4013	1.4053	0.3
12500	11875	13125	—	—	1.4015	1.4055	2.0
30000	27000	33000	0.969	0.977	1.4010	1.4100	2.0

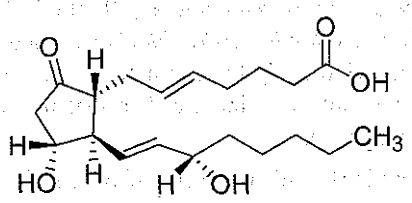
Dimethicone intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial Endotoxin (2.2.3). Not more than 10 Endotoxin Units per ml of the Dimethicone.

Storage. Store protected from moisture.

Labeling. Label it to indicate its nominal viscosity value. Dimethicone intended for use in coating containers that come in contact with articles for parenteral use is so labelled.

Dinoprostone



$C_{20}H_{32}O_5$ Mol Wt. 352.5

Dinoprostone is (Z)-7-[(1R,2R,3R)-3-hydroxy-2-[(E)-(3S)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (PGE_2).

Dinoprostone contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{20}H_{32}O_5$, calculated on the anhydrous basis.

Category. Prostaglandin E_2(PGE_2); Abortifacient; uterine stimulant.

Description. A white or almost white, crystalline powder or colourless crystals.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dinoprostone IPRS* or with the reference spectrum of dinoprostone.

Tests

Specific optical rotation (2.4.22). −90° to −82°, determined in a 0.5 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution (a). Dissolve 10 mg of the substance under examination in 2.0 ml of *methanol (58 per cent)*.

Test solution (b). Dissolve 20 mg of the substance under examination in 20.0 ml of *methanol (58 per cent)*.

Reference solution (a). A solution containing each of 0.004 per cent w/v each of *dinoprostone IPRS* and *dinoprostone impurity C IPRS* in *methanol (58 per cent)*.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 200.0 ml with *methanol (58 per cent)*.

Reference solution (c). Prepare *in situ* degradation compounds (dinoprostone impurity D and dinoprostone impurity E) by dissolving 1 mg of the substance under examination in 100 µl of *1M sodium hydroxide* (solution becomes brownish-red), wait for 4 minutes, add 150 µl of *1 M acetic acid* (yellowish-white opalescent solution) and dilute to 5.0 ml with *methanol (58 per cent)*.

Reference solution (d). A 0.1 per cent w/v solution of *dinoprostone IPRS* in *methanol (58 per cent)*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica,
- mobile phase: a mixture of 42 volumes of a 0.2 per cent v/v of *acetic acid*, and 58 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Dinoprostone (Retention time: about 18 minutes)	1.0	—
Dinoprostone impurity C[1]	1.2	—
Dinoprostone impurity D[2]	1.8	0.2
Dinoprostone impurity E[3]	2.0	0.7

[1](E)-7-[(1R, 2R, 3R)-3-hydroxy-2-[(E)-(3S)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid,

[2](Z)-7-[(1R,2S)-2-[(E,3S)-3-hydroxyoct-1-enyl]-5-oxocyclopent-3-en-1-yl]hept-5-enoic acid (prostaglandin A_2),

[3](Z)-7-[2-[(E,3S)-3-hydroxyoct-1-enyl]-5-oxocyclopenten-1-yl]hept-5-enoic acid (prostaglandin B_2).

Inject reference solution (a). The test is not valid unless the resolution between dinoprostone and dinoprostone impurity C is not less than 3.8

Inject reference solution (b), (c) and test solution (a). In the chromatogram obtained with the test solution (a), the area of any peak corresponding to dinoprostone impurity C is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent), the area of any peak corresponding to dinoprostone impurity D is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any peak corresponding to dinoprostone impurity E is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than the areas of other than the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

If any peak with a relative retention time to dinoprostone of about 0.8 is more than 0.5 per cent or if the total of other impurities is more than 1.0 per cent, record the chromatogram of the test solution (a) with a detector set at 230 nm. If the area of the peak at 230 nm is twice the area of the peak at 210 nm, multiply the area at 210 nm by 0.2 (correction factor for (*Z*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-5-oxo-2-[(*E*)-3-oxooct-1-enyl] cyclopentyl]hept-5-enoic acid (dinoprostone impurity F)).

Water (2.3.43). Not more than 0.5 per cent.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (d) and test solution (b).

Calculate the content of $C_{20}H_{32}O_5$.

Storage. Store at a temperature not exceeding -15°.

Dinoprostone Oral Solution

Dinoprostone Oral Solution is a solution of dinoprostone in a suitable aqueous vehicle.

Dinoprostone Oral Solution contains not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of dinoprostone, $C_{20}H_{32}O_5$.

Identification

A. Evaporate about 20.0 ml of the Oral Solution to 15.0 ml under a stream of nitrogen, extract the remaining solution with two 5 ml quantities of *chloroform*, combine the *chloroform* extracts and evaporate almost to dryness using a rotary evaporator or filter the *chloroform* solution through *anhydrous sodium sulphate*. Evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dinoprostone IPRS* or with the reference spectrum of dinoprostone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dilute a volume of the oral solution containing 10 mg of Dinoprostone to 100.0 ml with *methanol (60 per cent v/v)*.

Reference solution (a). Dilute 1.0 ml of test solution to 10.0 ml with *methanol (60 per cent v/v)* and further dilute 1 ml to 10.0 ml with *methanol (60 per cent. v/v)*

Reference solution (b). Dissolve 1 mg of *dinoprostone IPRS* in 100 µl of *1 M sodium hydroxide* (solution becomes brownish-red), allow to stand for 4 minutes and then add 150 µl of *1 M acetic acid* (a yellowish-white opalescent solution is produced). Dilute the above solution to 5 ml with *methanol (60 per cent v/v)* and further dilute 1.0 ml to 10.0 ml with *methanol (60 per cent v/v)*. The solution contains a mixture of prostaglandin A_2 (dinoprostone impurity D) and prostaglandin B_2 (dinoprostone impurity E).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Kromasil C18),
- mobile phase: a mixture of 40 volumes of 1.0 per cent v/v solution of *triethylamine*, adjusted to pH 2.3 with *orthophosphoric acid*, and 60 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 200 µl.

Name	Relative retention time	Correction factor
Dinoprostone (Retention time: about 22 minutes)	1.0	—
Dinoprostone impurity D[1]	1.7	0.2
Dinoprostone impurity E[2]	1.8	—

[1](Z)-7-[(1R,2S)-2-[(E,3S)-3-hydroxyoct-1-enyl]-5-oxocyclopent-3-en-1-yl]hept-5-enoic acid,

[2] (Z)-7-[2-[(E,3S)-3-hydroxyoct-1-enyl]-5-oxocyclopenten-1-yl]hept-5-enoic acid.

Inject reference solution (b). The test is not valid unless the resolution between dinoprostone impurity D and dinoprostone impurity E is at least 1.5.

Inject reference solution (a) and the test solution. The area of any peak due to dinoprostone impurity D is not more than 5 times the area of the prinicipal peak in the chromatogram obtained with reference solution (a) (5 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Use chromatographic system, test solution as described under Related substances with 20 µl as injection volume.

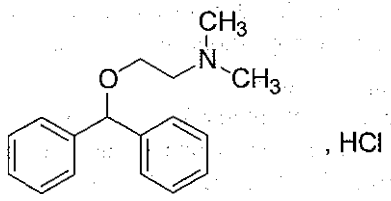
Reference solution. A 0.01 per cent w/v solution of *dinoprostone IPRS* in *methanol (60 per cent v/v)*.

Inject the reference solution and the test solution.

Storage. Store protected from light in a refrigerator (2° to 8°). Do not freeze.

Labelling. The label states the preparation is to be diluted before use and any oral solution not used within 7 days of the date of preparation should be discarded.

Diphenhydramine Hydrochloride



$C_{17}H_{21}NO,HCl$ Mol. Wt. 291.8

Diphenhydramine Hydrochloride is 2-benzhydryloxyethyldimethylamine hydrochloride.

Diphenhydramine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{21}NO,HCl$, calculated on the dried basis.

Category. Histamine H_1-receptor antagonist.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diphenhydramine hydrochloride IPRS* or with the reference spectrum of diphenhydramine hydrochloride. Examine the substances as discs prepared using *potassium chloride IR*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.025 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 253 nm, 258 nm and 264 nm; absorbance at about 253 nm, about 0.31, at about 258 nm, about 0.38 and at about 264 nm, about 0.3.

C. To 0.05 ml of a 5 per cent w/v solution add 2 ml of *sulphuric acid*; an intense yellow colour develops which changes to red on the addition of 0.5 ml of *nitric acid*. Add 15 ml of *water*, cool, add 5 ml of *chloroform* and shake; an intense violet colour develops in the chloroform layer.

D. It gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 4.0 to 6.0, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 70 mg of the substance under examination in 20.0 ml of the mobile phase. Dilute 2.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *2-(diphenylmethoxy)-N-methylethanamine IPRS (diphenhydramine impurity A IPRS)* in the mobile phase and dilute to 10.0 ml with the mobile phase. To 2.0 ml of the solution add 1.5 ml of the test solution and dilute to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of 0.54 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The relative retention time with reference to diphenhydramine (retention time is about 6 minutes) for diphenhydramine impurity A is about 0.9, for 2-[(*RS*)-(4-methylphenyl) phenylmethoxy]-*N,N*- dimethylethanamine (diphenhydramine

impurity B) is about 1.5, for 2-[(*RS*)-(4-bromophenyl) phenylmethoxy]-*N,N*- dimethylethanamine (diphenhydramine impurity C) is about 1.8, for benzhydrol (diphenhydramine impurity D) is about 2.6 and for benzophenone (diphenhydramine impurity E) is about 5.1. The correction factor for diphenhydramine impurity D is 0.7.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to diphenhydramine and diphenhydramine impurity A is not less than 2.0.

Inject reference solution (a) and the test solution. Run the chromatogram seven times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of peak due to diphenhydramine impurity A is not more than the area the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any secondary peak is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.250 g in 50 ml of *ethanol*, add 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining end-point potentiometrically (2.4.25). Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02918 g of $C_{17}H_{22}ClNO$.

Storage. Store protected from light.

Diphenhydramine Capsules

Diphenhydramine Hydrochloride Capsules

Diphenhydramine Capsules contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of diphenhydramine hydrochloride, $C_{17}H_{21}NO,HCl$.

Usual strengths. 25 mg; 50 mg.

Identification

Extract a quantity of the contents of the capsules containing 0.1 g of Diphenhydramine Hydrochloride with two quantities, each of 15 ml, of *chloroform*. Evaporate the combined extracts to dryness on a water-bath and dry the residue at 80° for 1 hour. The residue melts at about 168° (2.4.21), and complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diphenhydramine hydrochloride IPRS* or with the reference spectrum of diphenhydramine hydrochloride. Examine the substances as discs prepared using *potassium chloride IR*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.025 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at about 253 nm, 258 nm and 264 nm; absorbance at about 253 nm, about 0.31, at about 258 nm, about 0.38 and at about 264 nm, about 0.3.

C. To 0.05 ml of a 5 per cent w/v solution add 2 ml of *sulphuric acid*; an intense yellow colour develops which changes to red on the addition of 0.5 ml of *nitric acid*. Add 15 ml of *water*, cool, add 5 ml of *chloroform* and shake; an intense violet colour develops in the chloroform layer.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 500 ml of *water*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), as described under Assay using the following modifications.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *diphenhydramine hydrochloride IPRS* in dissolution medium to obtain a solution having a concentration similar to that of the test solution.

– injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{21}NO,HCl$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{21}NO,HCl$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 80 volumes of *chloroform*, 20 volumes of *methanol* and 1 volume of *diethylamine*.

Test solution. Shake a quantity of the contents of the capsules containing 100 mg of Diphenhydramine Hydrochloride with three quantities, each of 10 ml, of *chloroform*, filter and evaporate the combined filtrate almost to dryness; dissolve the residue in 5 ml of *chloroform*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *chloroform*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air for 5 minutes, spray with *sulphuric acid* and heat at 120° for 10 minutes until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 35 volumes of *acetonitrile* and 65 volumes of mobile phase A.

Test solution. Weigh and mix the contents of 20 capsules. Disperse a quantity of the mixed content containing 70 mg of Diphenhydramine Hydrochloride in *water* and dilute to 100.0 ml with *water*, mix and filter. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.01 per cent w/v, each of, *diphenhydramine related compound A (2-(Diphenylmethoxy)-N-methylethanamine hydrochloride) IPRS* and *diphenhydramine hydrochloride IPRS* in the solvent mixture.

Reference solution (b). A 0.007 per cent w/v solution of *diphenhydramine hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.54 per cent w/v solution of *monobasic potassium phosphate* in *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
4	65	35
7	20	80
9	65	35
13	65	35

The relative retention time with reference to diphenhydramine for diphenhydramine related compound A is about 0.9.

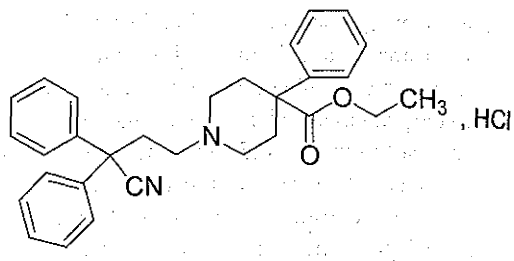
Inject reference solution (a). The test is not valid unless the resolution between the peaks due to diphenhydramine and diphenhydramine related compound A is not less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{17}H_{21}NO$, HCl in the capsules.

Storage. Store protected from moisture.

Diphenoxylate Hydrochloride



$C_{30}H_{32}N_2O_2,HCl$ Mol. Wt. 489.1

Diphenoxylate Hydrochloride is ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylpiperidine-4-carboxylate hydrochloride.

Diphenoxylate Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{30}H_{32}N_2O_2,HCl$, calculated on the dried basis.

Category. Antidiarrhoeal.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *diphenoxylate hydrochloride IPRS* or with the reference spectrum of diphenoxylate hydrochloride.

B. Dissolve about 30 mg in 5 ml of *methanol*, add 0.25 ml of *nitric acid* and 0.4 ml of *silver nitrate solution*. Shake and allow to stand; a curdled precipitate is formed. Centrifuge and rinse the precipitate with three quantities, each of 2 ml, of *methanol*. Carry out this operation rapidly in subdued light. Suspend the precipitate in 2 ml of *water* and add 1.5 ml of *10 M ammonia*; the precipitate dissolves easily.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *dichloromethane* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and mobile phase A.

Test solution. Dissolve 25 mg of the substance under examination in 20 ml of the solvent mixture with the aid of ultrasound and dilute to 25.0 ml with the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 900 ml of *water*, adjusted to pH 2.3 with *orthophosphoric acid* and dilute to 1000.0 ml with *water*,

B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
5	75	25
40	15	85
45	75	25

The relative retention time with reference to diphenoxylate (retention time: about 16 minutes) for diphenoxylate impurity A (diphenoxylic acid) is about 0.8.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to diphenoxylate impurity A is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.4 g in 40 ml of *ethanol (95 per cent)* and add 5 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Read the volume added between the two points of inflection.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.04891 g of $C_{30}H_{32}N_2O_2,HCl$.

Storage. Store protected from light.

Diphenoxylate Hydrochloride and Atropine Sulphate Tablets

Diphenoxylate Hydrochloride and Atropine Sulphate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of diphenoxylate hydrochloride, $C_{30}H_{32}N_2O_2,HCl$ and not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

Usual strength. Diphenoxylate Hydrochloride, 2.5 mg and Atropine Sulphate, 0.025 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to *atropine* and *diphenoxylate* in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 500 ml of *0.2 M acetic acid*,
Speed and time. 150 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. A 0.025 per cent w/v solution of *diphenoxylate hydrochloride IPRS* in *methanol*. Dilute further with dissolution medium to obtain a solution of known concentration similar to the concentration of the test solution.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with phenyl group (5 µm),
- mobile phase: a mixture of 65 volumes of *acetonitrile* and 35 volumes of a buffer solution prepared by

dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{30}H_{32}N_2O_2,HCl$ in the tablet.

Q. Not less than 75 per cent of the stated amount of $C_{30}H_{32}N_2O_2,HCl$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse one tablet in 15.0 ml of solvent mixture with the aid of ultrasound for about 15 minutes, allow to cool and dilute to 25.0 ml with solvent mixture and filter. Dilute further, if necessary.

Calculate the content of $C_{30}H_{32}N_2O_2,HCl$ and $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile*.

Buffer solution A. prepared by dissolving 192 mg of *sodium 1-pentanesulphonate monohydrate* to 200.0 ml with *water*, sonicate to dissolve, add 800 ml of *water* and 1.0 ml of *orthophosphoric acid*.

Buffer solution B. prepared by dissolving 192 mg of *sodium 1-pentanesulphonate monohydrate* to 200.0 ml with *water*, sonicate to dissolve, add 800 ml of *acetonitrile* and add 1.0 ml of *orthophosphoric acid* and mix.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 25 mg of *diphenoxylate hydrochloride* to a 250-ml volumetric flask, add approximately 100.0 ml of solvent mixture, and shake by mechanical means for at least 15 minutes. Sonicate for an additional 15 minutes, cool and dilute with solvent mixture to volume, and mix. Filter the solution, discarding the first few ml of the filtrate.

Reference solution (a). A 0.004 per cent solution of *atropine sulphate IPRS* in solvent mixture.

Reference solution (b). Transfer about 20 mg of *diphenoxylate hydrochloride IPRS* to a 200-ml volumetric flask, add about 100.0 ml of solvent mixture, and sonicate to dissolve. Add accurately measured 5.0 ml of reference solution (a), mix and cool. Then dilute with solvent mixture to volume. This solution contains about 0.1 mg of *diphenoxylate hydrochloride* and about 0.001 mg of *atropine sulphate* per ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with nitrile group bonded to porous silica (5 µm),
- mobile phase: a mixture of 34 volumes of buffer solution A and 66 volumes of buffer solution B,
- flow rate: 1.7 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

The relative retention time for atropine peak is about 0.35 with reference to diphenoxylate peak.

Inject reference solution (b). The test is not valid unless the resolution between atropine peak and diphenoxylate peak is not less than 5.0, the tailing factor is not more than 1.5 for atropine peak and the relative standard deviation for replicate injections is not more than 2.0 per cent for diphenoxylate peak and not more than 5.0 per cent for atropine peak.

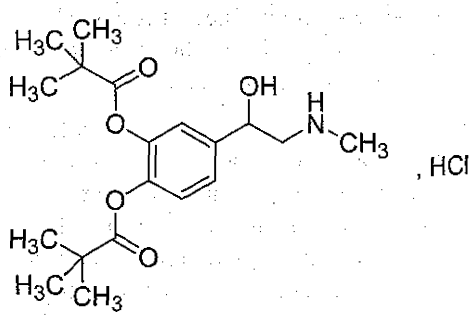
NOTE — If a significant tailing of the diphenoxylate peak is observed more than 2.5, it is recommended to maintain the column temperature at 25°, to stabilize the system.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{30}H_{32}N_2O_2,HCl$ and $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$ in the tablets.

Storage. Store protected from light and moisture.

Dipivefrine Hydrochloride



$C_{19}H_{29}NO_5,HCl$ Mol. Wt. 387.9

Dipivefrine Hydrochloride is (*RS*)-4-[1-Hydroxy-2-(methylamino)ethyl]-*o*-phenylene dipivalate hydrochloride.

Dipivefrine Hydrochloride contains not less than 97.5 per cent and not more than 102.0 per cent of $C_{19}H_{29}NO_5,HCl$, calculated on the dried basis.

Category. Miotic.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dipivefrine hydrochloride IPRS* or with the reference spectrum of dipivefrine hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

Impurities A and B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in *0.01 M hydrochloric acid* and dilute to 10.0 ml with *0.01 M hydrochloric acid.*

Reference solution. A solution containing 0.001 per cent w/v each of *adrenaline* and *adrenalone hydrochloride* in *0.01 M hydrochloric acid. (NOTE—Protect this solution from light).*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane amorphous organosilica polymer (5 µm),
- mobile phase: A. a 0.1 per cent v/v solution of *anhydrous formic acid*,
  B: a mixture of 40 volumes of *methanol* and 60 volumes *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
3	100	0
5	40	60
30	40	60
32	100	0

Name	Retention time
Dipivefrine impurity A[1]	2.2
Dipivefrine impurity B[2]	3.2

[1](±)-adrenaline),

[2]adrenalone.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to dipivefrine impurities A and B is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any impurity due to dipivefrine impurities A and B is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *methanol* and 60 volumes of *acetonitrile.* Add 55.0 ml of this mixture and 45.0 ml of *0.01 M hydrochloric acid.*

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). A 0.01 per cent w/v solution of *dipivefrine hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane amorphous organosilica polymer (5 µm),
- mobile phase: a mixture of 45 volumes of a 0.27 per cent w/v of *ammonia,* adjusted to pH 10.0 with *dilute acetic acid* and 55 volumes of a mixture of 40 volumes of *methanol* and 60 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Dipivefrine impurity C[1]	0.4	0.5
Dipivefrine impurity D[2]	0.4	0.5
Dipivefrine (Retention time: about 7 minutes)	1.0	—
Dipivefrine impurity E[3]	1.3	0.06
Dipivefrine impurity F[4]	2.0	—

[1]2-hydroxy-5-[(1*RS*)-1-hydroxy-2-(methylamino)ethyl]phenyl 2,2-dimethylpropanoate,

[2]2-hydroxy-4-[(1*RS*)-1-hydroxy-2-(methylamino)ethyl]phenyl 2,2-dimethylpropanoate,

[3]4-[(methylamino)acetyl]-1,2-phenylene bis(2,2-dimethylpropanoate),

[4] 4-[(1*RS*)-2-(ethylmethylamino)-1-hydroxyethyl]-1,2-phenylene bis
(2,2-dimethylpropanoate).

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject referene solution (a) and the test solution. In the chromatogram obtained with the test solution, the sum of areas of the peaks due to dipivefrine impurities C and D is not more than 0.3 times the area of principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent) and any peak with a mass distribution ratio less than 0.5.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° for 6 hours.

Assay. Determine by liquid chromatography (2.4.14) as described under test for Related substances with the following modification.

- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the tailing factor for the principle peak is not more than 2.0.

Inject reference solution (a) and (b).

Calculate the content of $C_{19}H_{30}ClNO_5$.

Dipivefrine Eye Drops

Dipivefrine Hydrochloride Eye Drops

Dipivefrine Eye Drops are a sterile solution of Dipivefrine Hydrochloride in purified water.

Dipivefrine Eye Drops contains not less than 90.0 per cent and not more than 110.0 per cent of dipivefrine hydrochloride, $C_{19}H_{29}NO_5,HCl$.

Usual strength. 0.1 per cent.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. a mixture of 1 volume of *formic acid*, 10 volumes of *methanol* and 30 volumes of *chloroform*.

Test solution. Dilute the eye drops with *water* to obtain a solution containing 0.1 per cent w/v of Dipivefrine Hydrochloride.

Reference solution. A 0.1 per cent w/v solution of *dipivefrine hydrochloride IPRS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with a mixture of 5 volumes of *ethylenediamine*, 45 volumes of *ethanol (95 per cent)* and 50 volumes of a 0.1 per cent w/v solution of *potassium hexacyanoferrate(III)* until saturated, allow to dry. Heat the plate at 100° for 10 minutes and examine in daylight.The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. The eye drops are clear (2.4.1).

pH (2.4.24). 2.5 to 3.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of eye drops with *water* to obtain a solution containing 0.1 per cent w/v of Dipivefrine Hydrochloride.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 1 volume of *glacial acetic acid*, 15 volumes of *0.014 M sodium dodecyl sulphate* and 24 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of eye drops with *water* to obtain a solution containing 0.1 per cent w/v of Dipivefrine Hydrochloride.

Reference solution. A 0.1 per cent w/v solution of *dipivefrine hydrochloride IPRS* in *water*.

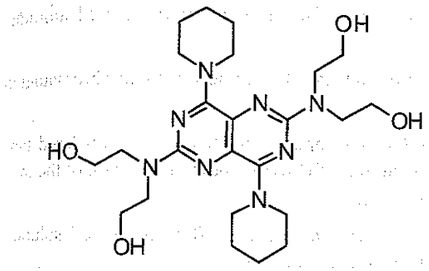
Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solutiona and the test solution.

Calculate the content of $C_{19}H_{29}NO_5,HCl$ in the eye drops.

Dipyridamole



$C_{24}H_{40}N_8O_4$ Mol. Wt. 504.6

Dipyridamole is 2,2',2'',2'''-[[4,8-Di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,6-diyl]dinitrilo]tetraethanol.

Dipyridamole contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{24}H_{40}N_8O_4$, calculated on the dried basis.

Category. Platelet aggregation inhibitor.

Description. A bright yellow, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *dipyridamole IPRS* or with the reference spectrum of dipyridamole.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Prepare the solutions immediately before use.

Test solution. Dissolve 0.1 g of the substance under examination in *methanol* and dilute to 50.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with *methanol*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 10 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: A. dissolve 1.0 g of *potassium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH 7.0 with *0.5 M sodium hydroxide* and dilute to 1000 ml with *water*,

  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	40	60
5	40	60
19	5	95
24	5	95
29	40	60

Name	Relative retention time	Correction factor
Dipyridamole impurity B[1]	0.2	1.7
Dipyridamole impurity F[2]	0.3	—
Dipyridamole impurity D[3]	0.9	—
Dipyridamole (Retention time: about 8 minutes)	1.0	—
Dipyridamole impurity E[4]	1.3	—
Dipyridamole impurity C[5]	1.6	—
Dipyridamole impurity A[6]	2.2	—

[1]2,2',2'',2''',2'''',2'''''[[8-(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,4,6-triyl]trinitrilo]hexaethanol,

[2]2,2',2'',2'''-[[4-[(2-hydroxyethyl)amino]-8-(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,6-diyl]dinitrilo] tetraethanol,

[3]2,2'-[[6-[(2-hydroxyethyl)amino]-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]nitrilo]diethanol,

[4]2,2',2'',2'''-[[6,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidine-2,4-diyl]dinitrilo]tetraethanol,

[5]2,2'-[[6-chloro-4,8-di(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]nitrilo]diethanol,

[6]2,2'-[[4,6,8-tri(piperidin-1-yl)pyrimido[5,4-*d*]pyrimidin-2-yl]nitrilo]diethanol.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to dipyridamole impurities A, B and C is

not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak corresponding to dipyridamole impurities D and E is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (2.3.12). To 1.25 g, add 10 ml of *water* and shake vigorously, filter and dilute to 15 ml with *water*, complies with limit test for chlorides (200 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.4 g in 70 ml of *methanol*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05046 g of $C_{24}H_{40}N_8O_4$.

Storage. Store protected from light.

Dipyridamole Tablets

Dipyridamole Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dipyridamole, $C_{24}H_{40}N_8O_4$.

Usual strengths. 25 mg; 75 mg; 100 mg.

Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Dipyridamole with 20 ml of *chloroform*, filter and evaporate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6), compare the spectrum with that obtained with *dipyridamole IPRS* or with the reference spectrum of dipyridamole.

B. To a quantity of the powdered tablets containing 10 mg of Dipyridamole add 50 ml of *methanol*, warm slightly, shake for 15 minutes and allow to cool and dilute to 100.0 ml with *methanol*, filter. To 10 ml of the filtrate add 1 ml of *1 M hydrochloric acid* and dilute to 100.0 ml with *methanol*. When examined in the range 220 nm to 450 nm (2.4.7), exhibits three maxima, at 230 nm, 285 nm and 405 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium: 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium. Measure the absorbance of the resulting solution at the maximum at 282 nm (2.4.7). Calculate the content of dipyridamole, $C_{24}H_{40}N_8O_4$ in the medium from the absorbance obtained from a solution of known concentration of *dipyridamole IPRS* in the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{24}H_{40}N_8O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Dipyridamole in 100.0 ml of the mobile phase and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of a 0.1 per cent w/v solution of *diltiazem hydrochloride IPRS* to 20.0 ml with reference solution (a).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 0.504 g of *potassium dihydrogen orthophosphate* in 370 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, add 80 ml of *acetonitrile* and 550 ml of *methanol*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume: 20 µl.

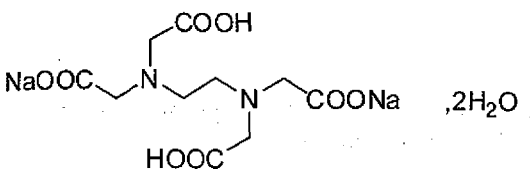
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to diltiazem and dipyridamole is not less than 2.0.

Inject reference solution (a) and the test solution. Run the chromatogram nine times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. To 10 whole tablets add 300 ml of *1 M hydrochloric acid*, heat at 40° for 20 minutes with shaking, allow to cool and dilute to 500 ml with *1 M hydrochloric acid*, filter and dilute, if necessary, with *1 M hydrochloric acid* to obtain a solution containing 0.05 per cent w/v of Dipyridamole. Dilute 1 volume to 50 volumes with *water* and measure the absorbance at the maximum at 283 nm (2.4.7). Dilute 1 volume of a 0.05 per cent w/v solution of *dipyridamole IPRS* in *1 M hydrochloric acid* to 50 volumes with *water*. Calculate the content of $C_{24}H_{40}N_8O_4$ in the tablets.

Disodium Edetate



$C_{10}H_{14}N_2Na_2O_8,2H_2O$ Mol. Wt. 372.2

Disodium Edetate is disodium ethylenediaminetetraacetate dihydrate.

Disodium Edetate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

Category. Pharmaceutical aid; chelating agent in metal poisoning.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disodium edetate IPRS* or with the reference spectrum of disodium edetate.

B. Dissolve 2 g in 25 ml of water, add 6 ml of *lead nitrate solution*, shake and add 3 ml of *potassium iodide solution*; no yellow precipitate is produced. Make alkaline to *red litmus paper* with *2 M ammonia* and add 5 ml of *ammonium oxalate solution*; no precipitate is produced.

C. Dissolve 0.5 g in 10 ml of *water*, add 0.5 ml of a 10 per cent w/v solution of *calcium chloride*, make alkaline to *red litmus paper* with *2 M ammonia* and add 3 ml of *ammonium oxalate solution*; no precipitate is produced.

D. It gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 4.0 to 5.5, determined in a 5.0 per cent w/v solution.

Impurity A. Determine by liquid chromatography (2.4.14).

NOTE—Carry out the test protected from light.

Solvent A. A 1.0 per cent w/v solution of *cupric nitrate* in *water*.

Test solution. Dissolve 100 mg of the substance under examination in 10.0 ml of solvent A with the aid of ultrasound and filter.

Reference solution (a). Transfer 100 mg of *nitrilotriacetic acid* to a 10-ml volumetric flask, add 0.5 ml of *ammonium hydroxide*, mix and dilute with *water* to volume.

Reference solution (b). Transfer 1 g of Disodium Edetate to a 100-ml volumetric flask, add 100 µl of reference solution (a), dilute with solvent A, dissolve with the aid of ultrasound and filter.

Reference solution (c). Transfer 10 mg of disodium edetate to a 100-ml volumetric flask, add 100 µl of reference solution (a), dilute with solvent A, dissolve with the aid of ultrasound and filter.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octylsilane bonded to porous silica (1.5 to 10 µm),
- mobile phase: dilute 10 ml of *1 M tetrabutylammonium hydroxide* in *methanol* to 200.0 ml with *water*, adjusted to pH 7.5 ± 0.1 with *dilute orthophosphoric acid*, add 90 ml of *methanol* and dilute to 1000 ml with *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

The relative retention time with reference to edetate for nitrilotriacetic acid and copper is about 0.35 and 0.65, respectively.

Inject reference solution (b) and (c). The test is not valid unless the resolution between the peaks due to nitrilotriacetic acid and copper is not less than 3.0 in the chromatogram obtained with reference solution (c) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of peak corresponding to nitrilotriacetic acid is not more than difference between the area of nitrilotriacetic acid peak obtained from reference solution (b) and the test solution (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Iron (2.3.14). 20 ml of a 2.5 per cent w/v solution complies with the limit test for iron (80 ppm). Add 0.25 g of *calcium chloride* to each solution before adding mercaptoacetic acid.

Assay. Dissolve 0.5 g in sufficient *water* to produce 300 ml and add 2 g of *hexamine* and 2 ml of *2 M hydrochloric acid*.

Titrate with *0.1 M lead nitrate* using about 50 mg of *xylenol orange triturate* as indicator until the colour of the indicator changes to brick red. Carry out a blank titration.

1 ml of *0.1 M lead nitrate* is equivalent to 0.03722 g of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

Disodium Edetate Injection

Disodium Edetate Injection is a sterile solution of Disodium Edetate in Water for Injections, containing varying amounts of the disodium and trisodium salts as a result of pH adjustment.

Disodium Edetate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of disodium edetate, $C_{10}H_{14}N_2Na_2O_8$.

Usual strengths. 3 g per 15 ml; 3 g per 20 ml.

Identification

To a volume containing about 3 g of Disodium Edetate add *3 M hydrochloric acid* to adjusted to pH 5.0 and evaporate to dryness on a steam-bath to dryness. The residue so obtained complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disodium edetate IPRS* or with the reference spectrum of disodium edetate.

B. Dissolve 2 g in 25 ml of *water*, add 6 ml of *lead nitrate solution*, shake and add 3 ml of *potassium iodide solution*; no yellow precipitate is produced. Make alkaline to *red litmus paper* with *2 M ammonia* and add 5 ml of *ammonium oxalate solution*; no precipitate is produced.

C. Dissolve 0.5 g in 10 ml of *water*, add 0.5 ml of a 10 per cent w/v solution of *calcium chloride*, make alkaline to *red litmus paper* with *2 M ammonia* and add 3 ml of *ammonium oxalate solution*; no precipitate is produced.

Tests

pH (2.4.24). 6.5 to 7.5.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of disodium edetate.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

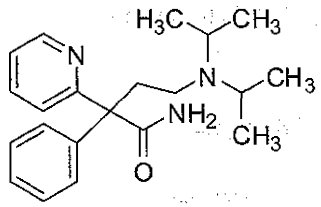
Assay. Dilute a measured volume containing about 0.6 g of Disodium Edetate with *water* to produce 100 ml, mix and add 2 g of *hexamine* and 2 ml of *2 M hydrochloric acid*. Titrate with *0.1 M lead nitrate* using about 50 mg of *xylenol orange triturate* as indicator until the colour of the indicator changes to brick red. Carry out a blank titration.

1 ml of *0.1 M lead nitrate* is equivalent to 0.03362 g of $C_{10}H_{14}N_2Na_2O_8$.

Storage. Store in single dose containers.

Labelling. The label states the strength in terms of anhydrous disodium edetate contained in a suitable dose-volume.

Disopyramide



$C_{21}H_{29}N_3O$ Mol. Wt. 339.5

Disopyramide is (*RS*)-4-(diisopropylamino)-2-phenyl-2-(pyridin-2-yl)butyramide.

Disopyramide contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{21}H_{29}N_3O$, calculated on the dried basis.

Category. Antiarrhythmic.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disopyramide IPRS* or with the reference spectrum of disopyramide. Examine the substances as discs prepared by placing 50 µl of a 5 per cent solution in *dichloromethane* on a disc of *potassium bromide*. Dry the discs at 60° for 1 hour before use.

B. When examined in the range 240 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in 0.5 per cent *methanolic sulphuric acid* shows an absorption maximum at about 269 nm and a shoulder at 263 nm ; specific absorbance at about 269 nm is 190 to 210.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane*.

Test solution (a). Dissolve 0.2 g of the substance under examination in *methanol* and dilute to 10.0 ml with the same solvent.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

Reference solution (a). A 0.2 per cent w/v solution of *disopyramide IPRS* in *methanol*.

Reference solution (b). Dilute 0.5 ml of test solution (b) to 20.0 ml with *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of warm air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 80° over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 2 hours.

Assay. Dissolve 0.13 g in 30 ml of *anhydrous acetic acid*, add 0.2 ml of *naphtholbenzein solution*. Titrate with *0.1 M perchloric acid* until the colour changes from yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01697 g of $C_{21}H_{29}N_3O$.

Storage. Store protected from light.

Disopyramide Capsules

Disopyramide Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of disopyramide, $C_{21}H_{29}N_3O$.

Usual strength. 200 mg.

Identification

A. Shake a quantity of the contents of the capsules containing about 0.2 g of Disopyramide with 50 ml of *chloroform* for 15 minutes, filter, evaporate the filtrate to dryness using a rotary evaporator and dissolve the residue in 2 ml of *chloroform*. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disopyramide IPRS* or with the reference spectrum of disopyramide.

B. When examined in the range 230 nm to 350 nm (2.4.7), the solution obtained in the assay shows an absorption maximum at about 269 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *18 M ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane*.

Test solution. Shake a quantity of the contents of the capsules containing about 0.2 g of Disopyramide with 20.0 ml of *methanol* for 30 minutes and filter.

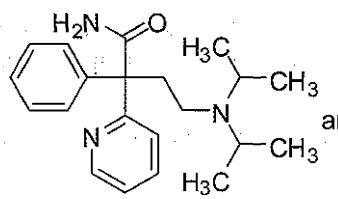
Reference solution. Dilute 1.0 ml of test solution to 400 ml with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in current of air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.25 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing 0.04 g of Disopyramide, add 40 ml of *0.05 M methanolic sulphuric acid*, shake for 15 minutes, dilute to 100.0 ml with the same solvent and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with *0.05 M methanolic sulphuric acid*. Measure the absorbance of the resulting solution at the maximum at about 269 nm (2.4.7). Calculate the content of $C_{21}H_{29}N_3O$ taking 198.5 as the specific absorbance at 269 nm.

Disopyramide Phosphate



and enantiomer, H_3PO_4

$C_{21}H_{32}N_3O_5P$ Mol. Wt. 437.5

Disopyramide Phosphate is (2*RS*)-4-[bis(1-methylethyl) amino]-2-phenyl-2-(pyridin-2-yl)butanamide dihydrogen phosphate.

Disopyramide Phosphate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{32}N_3O_5P$, calculated on the dried basis.

Category. Antiarrhythmic.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disopyramide phosphate IPRS* or with the reference spectrum of disopyramide phosphate.

B. When examined in the range 240 nm to 350 nm (2.4.7), a 0.005 per cent w/v solution in *0.05 M methanolic sulphuric acid* shows absorption maxima at about 269 nm and a shoulder at 263 nm; specific absorbance at the maximum is 147 to 163.

C. In the Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in chromatogram obtained with reference solution (a). Spray with *dilute potassium iodobismuthate solution* and examine in day light. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in chromatogram obtained with reference solution (a).

D. Solution A gives reaction (A) of phosphates (2.3.1).

Tests

Solution A. A 5.0 per cent w/v solution in *carbon dioxide-free water.*

Appearance of solution. Solution A is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 4.0 to 5.0, determined in solution A.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 1 volume of *concentrated ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane.*

Test solution (a). Dissolve 0.2 g of the substance under examination in *methanol* and dilute to 10.0 ml with the same solvent.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol.*

Reference solution (a). A 0.2 per cent w/v solution of *disopyramide phosphate IPRS* in *methanol.*

Reference solution (b). Dilute 0.5 ml of test solution (b) to 20.0 ml with *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.180 g in 30 ml of *anhydrous acetic acid*, add 0.2 ml of *naphtholbenzein solution*. Titrate with *0.1 M perchloric acid* until the colour changes from yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02188 g of $C_{21}H_{32}N_3O_5P$.

Storage. Store protected from light.

Disopyramide Phosphate Prolonged-release Capsules

Disopyramide Phosphate Sustained-release Capsules; Disopyramide Phosphate Extended-release Capsules

Disopyramide Phosphate Prolonged-release Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of disopyramide, $C_{21}H_{29}N_3O$.

Usual strengths. 100 mg; 150 mg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 85 volumes of *toluene*, 14 volumes of *absolute ethanol* and 1 volume of *ammonium hydroxide.*

Test solution. Transfer a quantity of the contents of the capsules containing about 195 mg of Disopyramide Phosphate to a 25-ml volumetric flask, add 20 ml of *methanol*, and shake for 20 minutes. Dilute with *methanol* to volume, mix, and filter.

Reference solution. A 0.77 per cent w/v solution of *disopyramide phosphate IPRS* in *methanol.*

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 1000 ml of a buffer solution prepared by dissolving 13.6 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *hydrochloric acid*,

Speed and time. 100 rpm and 1 hour, 2 hours, 5 hours, 12 hours.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the

filterate, suitably diluted with the dissolution medium if necessary at 261 nm. Calculate the content of disopyramide phosphate, $C_{21}H_{29}N_3O$ in the medium from the absorbance obtained from a solution of known concentration of *disopyramide phosphate IPRS*.

Q. Not less than 5 per cent and not more than 25 per cent in 1 hour, not less than 17 per cent and not more than 43 per cent in 2 hours, not less than 50 per cent and not more than 80 per cent in 5 hours, and not less than 85 per cent in 12 hours, of the stated amount of $C_{21}H_{29}N_3O$.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 650 mg of Disopyramide Phosphate to a 500-ml volumetric flask, add about 400 ml of *0.1 M sulphuric acid*, and shake for 30 minutes. Dilute with *0.1 M sulphuric acid* to volume, mix, and filter. Dilute further with *0.1 M sulphuric acid* to get a final concentration of about 40 µg per ml and measure the absorbance of the resulting solution at the maximum at about 261 nm (2.4.7).

Calculate the content of $C_{21}H_{29}N_3O$ from the absorbance of a solution of known concentration of *disopyramide phosphate IPRS*.

Disopyramide Phosphate Capsules

Disopyramide Phosphate Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of disopyramide, $C_{21}H_{29}N_3O$.

Usual strength. 150 mg.

Identification

Suspend a quantity of the contents of the capsules containing about 0.2 g of disopyramide in 50 ml of *chloroform*, add 2 ml of *13.5 M ammonia*, shake and filter through *anhydrous sodium sulphate*. Evaporate the filtrate to dryness using a rotary evaporator. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disopyramide IPRS* or with the reference spectrum of disopyramide.

B. When examined in the range 230 nm to 350 nm (2.4.7), the solution obtained in the Assay shows absorption maximum only at about 269 nm and a shoulder at 263 nm.

C. Shake a quantity of the contents of the capsules containing about 0.4 g of disopyramide with 20 ml of *water* and filter. The filtrate gives the reactions of phosphates (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*;

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 262 nm (2.4.7). Calculate the content of $C_{21}H_{29}N_3O$ in the medium taking 125 as the specific absorbance at 262 nm.

Q. Not less than 70 per cent of the stated amount of $C_{21}H_{29}N_3O$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *18 M ammonia*, 30 volumes of *acetone* and 30 volumes of *cyclohexane*.

Test solution. Shake a quantity of the contents of the capsules containing 0.2 g of disopyramide with 20.0 ml of *methanol* for 30 minutes and filter.

Reference solution. Dilute 1.0 ml of test solution to 200.0 ml with *methanol*.

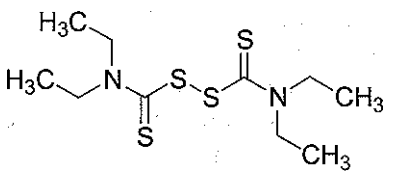
Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 0.04 g of Disopyramide, add 40 ml of *0.05 M methanolic sulphuric acid*, shake for 15 minutes, dilute to 100.0 ml with the same solvent and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with *0.05 M methanolic sulphuric acid*. Measure the absorbance of the resulting solution at the maximum at 269 nm (2.4.7). Calculate the content of $C_{21}H_{29}N_3O$ taking 198.5 as the specific absorbance at 269 nm.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of disopyramide.

Disulfiram



$C_{10}H_{20}N_2S_4$ Mol.Wt. 296.5

Disulfiram is thioperoxydicarbonic diamide[$(H_2N)C(S)]_2S_2$, tetraethyl-; Bis(diethylthiocarbamoyl) disulfide.

Disulfiram contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{10}H_{20}N_2S_4$.

Category. Used in the treatment of alcoholism.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disulfiram IPRS* or with the reference spectrum of disulfiram.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Disperse 0.2 g of Disulfiram in *methanol* and dilute to 100.0 ml with *methanol*. Dilute 10.0 ml of the solution to 20.0 ml with the solvent mixture.

Reference solution (a). A 0.01 per cent w/v solution of *disulfiram IPRS* in *methanol*. Dilute 10.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.005 per cent w/v of *disulfiram IPRS* and 0.001 per cent w/v of *sulfiram IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 4°,
- mobile phase: A. a buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 7.0 with 45 per cent w/v solution of *potassium hydroxide*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 15 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
8	30	70
12	30	70
12.1	60	40
16	60	40

Name	Relative retention time	Correction factor
Diethyldithiocarbamic acid	0.18	0.83
Tetraethylthiourea[1]	0.69	0.91
Sulfiram[2]	0.80	1.82
Disulfiram	1.0	—

[1]1,1,3,3-Tetraethylthiourea,

[2]Diethylthiocarbamic thioanhydride.

Inject reference solution (b). The test is not valid unless the resolution between sulfiram and disulfiram is not less than 8.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to diethyldithiocarbamic acid, tetraethylthiourea and sulfiram, each of, is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Selenium. Not more than 30 ppm, using 0.2 g. Determine by the Oxygen-Flask method (2.3.34).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Test solution. Dissolve 50 mg of the substance under examination in 40 ml of *ethanol*, with the aid of ultrasound for 5 minutes and dilute to 50.0 ml with *ethanol*. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *disulfiram IPRS* in *ethanol*. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 7.0 with 45 per cent w/v solution of *potassium hydroxide* and 70 volumes of *methanol*,

- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1800theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{20}N_2S_4$.

Storage. Store protected from light and moisture.

Disulfiram Tablets

Disulfiram Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of disulfiram $C_{10}H_{20}N_2S_4$.

Usual strengths. 200 mg; 250 mg; 500 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.5 g of Disulfiram with 20 ml of *methanol*, filter, evaporate the filtrate to dryness and dry at 105°. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *disulfiram IPRS* or with the reference spectrum of disulfiram.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Disintegration (2.5.1). Not more than 15 minutes, carry out the test without discs.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Disperse a quantity of the powdered tablets containing 0.2 g of Disulfiram in *methanol* and dilute to 100.0 ml with *methanol*. Dilute 10.0 ml of the solution to 20.0 ml with the solvent mixture.

Reference solution (a). A 0.01 per cent w/v solution of *disulfiram IPRS* in *methanol*. Dilute 10.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.005 per cent w/v of *disulfiram IPRS* and 0.001 per cent w/v of *sulfiram IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 4°,
- mobile phase: A. a buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 7.0 with 45 per cent w/v solution of *potassium hydroxide*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 15 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
8	30	70
12	30	70
12.1	60	40
16	60	40

Name	Relative retention time	Correction factor
Diethyldithiocarbamic acid	0.18	0.83
Tetraethylthiourea[1]	0.69	0.91
Sulfiram[2]	0.80	1.82
Disulfiram	1.0	---

[1]1,1,3,3-Tetraethylthiourea,

[2]Diethylthiocarbamic thioanhydride.

Inject reference solution (b). The test is not valid unless the resolution between sulfiram and disulfiram is not less than 8.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to diethyldithiocarbamic acid, tetraethylthiourea and sulfiram, each of, is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of Disulfiram in 70 ml of *ethanol*, with the aid of ultrasound with intermittent shaking for 30 minutes, dilute to 100.0 ml with *ethanol* and filter. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *disulfiram IPRS* in *ethanol*. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 7.0 with 45 per cent w/v solution of *potassium hydroxide* and 70 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 20 µl.

Run the chromatogram 1.5 times the retention time of disulfiram.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

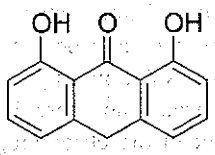
Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{20}N_2S_4$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Dithranol

Anthralin; Dioxyanthranol



$C_{14}H_{10}O_3$ Mol. Wt. 226.2

Dithranol is 1,8-dihydroxyanthrone.

Dithranol contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{14}H_{10}O_3$, calculated on the dried basis.

Category. Topical antipsoriatic.

Description. A yellow or orange-yellow, microcrystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dithranol IPRS* or with the reference spectrum of dithranol.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *chloroform* shows absorption maxima at about 255 nm, 287 nm and 354 nm; absorbances at the maxima, about 0.55, 0.5 and 0.45 respectively.

C. Melts at about 178° (2.4.21).

Tests

Dihydroxyanthracene. Dissolve 0.1 g in 5 ml of hot *benzene*; a clear yellow or orange solution is produced.

Dihydroxyanthraquinone. Dissolve 1 mg in a few drops of *sulphuric acid*; a clear orange solution with no trace of violet colour is produced.

Related substances. A. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in 20 ml of *dichloromethane*, add 1.0 ml of *glacial acetic acid* and dilute to 100.0 ml with *hexane*.

Reference solution. A solution containing 0.1 per cent w/v each of dithranol impurity A (*anthrone*), dithranol impurity B (*dantron*), *dithranol impurity C IPRS (4,4',5,5'-tetrahydroxy-9,9'-bianthracenyl-10,10'(9H,9'H)-dione IPRS)* and *dithranol IPRS* in *dichloromethane*. To 1.0 ml of the solution, add 19.0 ml of *dichloromethane* and 1.0 ml of *glacial acetic acid* and dilute to 50.0 ml with *hexane*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with silica (5 µm),
- mobile phase: a mixture of 1 volume of *glacial acetic acid*, 5 volumes of *dichloromethane* and 82 volumes of *hexane*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

The elution order of the peaks is dithranol, dithranol impurity B, dithranol impurity A and dithranol impurity C.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to dithranol and dithranol impurity B is not less than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 1.5 times the retention time of dithranol impurity C. In the chromatogram obtained with the test solution, the area of peak corresponding to dithranol impurities A, B and C is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (1.0 per cent).

B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 5 ml of *tetrahydrofuran* and dilute to 25.0 ml with the mobile phase.

Reference solution. Dissolve 5 mg each of *dithranol impurity D IPRS (1-hydroxyanthracen-9(10H)-one IPRS)* and *dithranol IPRS* in 5 ml of *tetrahydrofuran* and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 2.5 volumes of *glacial acetic acid,* 40 volumes of *tetrahydrofuran* and 60 volumes of *water,*
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to dithranol impurity D and dithranol is not less than 2.5.

Inject the reference solution and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to dithranol impurity D is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (2.5 per cent) and the sum of areas of all the secondary peaks of tests A and B is not more than 3.0 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.2 g in 50 ml of *anhydrous pyridine*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02262 g of $C_{14}H_{10}O_3$.

Storage. Store protected from light.

Dithranol Ointment

Dithranol Ointment contains Dithranol, in fine powder, in a suitable base.

Dithranol Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dithranol, $C_{14}H_{10}O_3$.

Usual strengths. 0.1 to 2 per cent w/w.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Heat a quantity containing 0.5 mg of Dithranol with 5 ml of *1 M sodium hydroxide* on a water-bath with constant stirring; a pink colour is produced in the aqueous layer.

Tests

Dihydroxyanthracene. Dissolve a quantity containing 0.1 g of Dithranol in 5 ml of hot *benzene*; a yellow or orange solution is produced.

Dihydroxyanthraquinone. Dissolve a quantity containing 1 mg of Dithranol in a few drops of *sulphuric acid*; an orange solution with no trace of violet colour is produced.

Other tests. Comply with the tests stated under Ointments.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the ointment containing 5 mg of Dithranol, disperse in 20 ml of *dichloromethane,* add 1.0 ml of *glacial acetic acid,* dilute to 100.0 ml with *hexane* and filter.

Reference solution. Add 1.0 ml of *glacial acetic acid* to 20.0 ml of a 0.025 per cent w/v solution of *dithranol IPRS* in *dichloromethane* and add sufficient *hexane* to produce 100.0 ml.

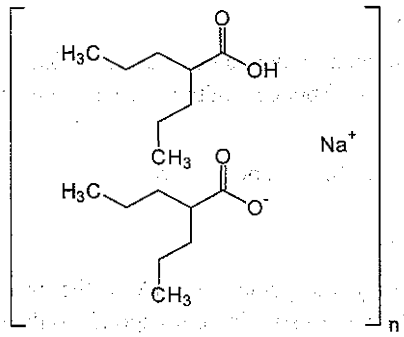
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (5 µm),
- mobile phase: a mixture of 82 volumes of *hexane,* 5 volumes of *dichloromethane* and 1 volume of *glacial acetic acid,*
- flow rate: 2 ml per minute,
- spectrophotometer set at 354 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{10}O_3$ in the ointment.

Storage. Store protected from light.

Divalproex Sodium



$(C_{16}H_{31}NaO_4)_n$ Mol. Wt. 310.4

Divalproex Sodium is 2-Propyl-pentanoic acid sodium salt (2:1). Sodium hydrogen bis(2-propylvalerate) oligomer.

Category. Anticonvulsant.

Divalproex Sodium contains not less than 98.0 per cent and not more than 102.0 per cent of available valproic acid, $C_8H_{16}O_2$.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *divalproex sodium IPRS* or with the reference spectrum of divalproex sodium.

B. Ignite about 100 mg, the residue gives the reaction of sodium (2.3.1).

Tests

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 1.0 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 50 mg of the substance under examination in the mobile phase and dilute to 100 ml with the same solvent.

Reference solution (a). A 0.5 per cent w/v solution of *valproic acid IPRS* in the mobile phase.

Reference solution (b). A 0.05 per cent w/v solution of *valproic acid impurity A IPRS (diallylacetic acid IPRS)* in *acetonitrile.*

Reference solution (c). Mix 10 ml of reference solution (a) and 1 ml of reference solution (b) and dilute to 100 ml with the mobile phase to obtain a solution having concentrations of 0.05 per cent w/v of valproic acid and 0.0005 per cent w/v of valproic acid impurity A.

Reference solution (d). Dilute a volume of reference solution (a) with the mobile phase to obtain a solution having a concentration of about 0.05 per cent w/v of valproic acid.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 3.5 g of *monobasic sodium phosphate monohydrate* in 900 ml of *water*, adjusted to pH 3.5 with *orthophosphoric acid* and diluted to 1000 ml with *water* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

The relative retention time with reference to valproic acid for valproic acid impurity A is about 0.69.

Inject reference solution (c).The test is not valid unless the resolution between the peaks due to valproic acid impurity A and valproic acid is not less than 5.0 and the tailing factor for valproic acid peak is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (d) and the test solution.

Calculate the content of available valproic acid, $C_8H_{16}O_2$ in the divalproex sodium taken by the formula:

$$100\,(C_S / C_U)\,(r_U / r_S)\,(310.41/144.21)\,(½)$$

in which C_S is the concentration, in mg per ml of valproic acid in the reference solution, C_U is the concentration of divalproex sodium in mg per ml in the test solution, r_U *and* r_S are the peak areas for valproic acid obtained from the test solution and the reference solution; 310.4 and 144.2 are the molecular weights for divalproex sodium repeating unit and valproic acid, respectively; and 2 is the number of moles of valproic acid per mole of divalproex sodium repeating unit.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Divalproex Gastro-resistant Tablets

Divalproex Tablets; Divalproex Sodium Gastro-resistant Tablets; Divalproex Sodium Tablets

Divalproex Gastro-resistant Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of valproic acid, $C_8H_{16}O_2$. They are made gastro-resistant by enteric-coating or by other means.

Usual strengths. 125 mg; 250 mg; 500 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

A. Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid,*

Speed and time. 50 rpm and 2 hours.

Withdraw the dissolution medium completely and transfer the tablets in 900 ml of *phosphate buffer 7.5* for test B.

B. Apparatus No. 2 *(Paddle),*

Medium. 900 ml of *phosphate buffer 7.5,*

Speed and time. 50 rpm and 1 hour.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, to obtain a solution of 0.012 per cent w/v in the dissolution medium.

Reference solution. A 0.012 per cent w/v solution of *valproic acid IPRS* in the dissolution medium.

NOTE—A volume of acetonitrile not exceeding 10 per cent may be used to dissolve.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm packed with phenyl group bonded to porous silica (4 µm),
- mobile phase: a mixture of 35 volumes of *citrate buffer,* 35 volumes of *potassium phosphate buffer and* 30 volumes of *acetonitrile*, adjusted to pH 3.0 with *orthophosphoric acid,*
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{16}O_2$ in the tablets.

Q. Not less than 80 per cent of the stated amount of $C_8H_{16}O_2$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 50 mg of valproic acid, disperse in 100.0 ml of the mobile phase.

Reference solution. A 0.05 per cent w/v solution of *valproic acid IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm packed with phenyl group bonded to porous silica (4 µm),
- mobile phase: a mixture of 70 volumes of *citrate buffer and* 30 volumes of *acetonitrile*, adjusted to pH 3.0 with *orthophosphoric acid,*
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{16}O_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Divalproex Prolonged-release Tablets

Divalproex Sodium Prolonged-release Tablets; Divalproex Sustained-release Tablets; Divalproex Extended-release Tablets

Divalproex Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Divalproex Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of valproic acid, $C_8H_{16}O_2$.

Usual strengths. 125 mg;250 mg; 500 mg; 750 mg; 1000 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by gas chromatography (2.4.13).

Internal standard solution (a). A 0.01 per cent w/v solution of *octanoic acid* in *dichloromethane.*

Internal standard solution (b). A 0.01 per cent w/v solution of *octanoic acid* in *0.1 M sodium hydroxide.*

Test solution (a). Transfer a weighed quantity of powdered tablets containing 500 mg of Divalproex Sodium into a 250-ml separating funnel. Add 20 ml of *water* and shake to form slurry. Acidify with *2 M sulphuric acid* until acidic to litmus. Extract thrice with 20 ml portion of *dichloromethane*. Collect the combined dichloromethane layers in separating funnel. Wash this layer with 15 ml *water*. Collect the dichloromethane layer. Wash the combined dichloromethane extract with 10 ml of *water*, shake with *anhydrous sodium sulphate*, filter and evaporate the filtrate to a volume of about 10 ml at a temperature not exceeding 30° using a rotary evaporator.

Test solution (b). Transfer a weighed quantity of powdered tablets containing 500 mg of Divalproex Sodium into a 250-ml separating funnel and 10 ml of internal standard solution (b) and shake to form slurry. Acidify with *2 M sulphuric acid* until acidic to litmus. Extract thrice with 20-ml portion of *dichloromethane*. Collect the combined dichloromethane layers in separating funnel. Wash this layer with 15 ml *water*. Collect the dichloromethane layer. Wash the combined dichloromethane extract with 10 ml of *water*, shake with *anhydrous sodium sulphate*, filter and evaporate the filtrate to a volume of about 10 ml at a temperature not exceeding 30° using a rotary evaporator.

Chromatographic system

- a fused-silica column 30 m x 0.53 mm, packed with macrogol 20000 2-nitroterephthalate (0.5 µm),
- temperature:
  column. 130° from 0 to 10 minutes, 130° to 190° at a constant rate of 3° per minute and hold at 190° for 15 minutes,
- inlet port at 220° and detector at 220°,
- a flame ionization detector,
- flow rate: 6.33 ml per minute using nitrogen as carrier gas.

Inject 1 µl of internal standard solution (a) and test solution (b). The test is not valid unless the resolution between the peaks due to valproic acid and internal standard (octanoic acid) is not less than 3.0.

Inject 1 µl of internal standard solution (a), test solution (a) and (b). In the chromatogram obtained with test solution (b) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the peak due to internal standard (octanoic acid) (0.5 per cent) and the area of any secondary peak is not more than the area of the peak due to internal standard (octanoic acid) (0.2 per cent). Ignore any peak due to dichloromethane and any peak coming after retention time 30 minutes.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 0.5 g of *citric acid monohydrate* and 0.4 g of *dibasic sodium phosphate* in 1000 ml of *water* adjusted to pH 2.0 with *orthophosphoric acid.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.25 g of valproic acid in 70 ml of *methanol,* with the aid of ultrasound for about 1 hour, dilute to 100.0 ml with *methanol* and filter. Dilute 4.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution. A 0.25 per cent w/v solution of *valproic acid IPRS* in *methanol.* Dilute 4.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with phenyl group bonded to porous silica (4 µm),
- mobile phase: a mixture of 11 volumes of *methanol* and 9 volumes of buffer solution prepared by dissolving 0.5 g of *citric acid monohydrate* and 0.4 g of *dibasic sodium phosphate* in 1000.0 ml of *water,* adjusted to pH 5.0 with *orthophosphoric acid,*
- flow rate: 0.7 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

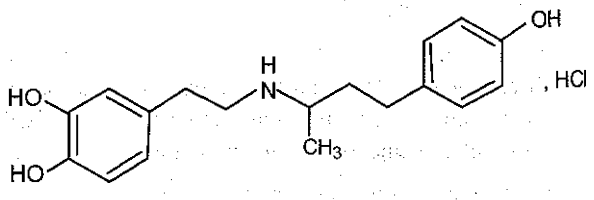
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of valproic acid, $C_8H_{16}O_2$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Dobutamine Hydrochloride



$C_{18}H_{23}NO_3,HCl$ Mol. Wt. 337.9

Dobutamine Hydrochloride is (*RS*)-4-[2-[[3-(4-hydroxyphenyl)-1-methylpropyl]amino]ethyl]benzene-1,2-diol hydrochloride.

Dobutamine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{23}NO_3,HCl$, calculated on the dried basis.

Category. Vasopressor.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dobutamine hydrochloride IPRS* or with the reference spectrum of dobutamine hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1), using a mixture of equal volumes of *methanol* and *water*.

Tests

Acidity or alkalinity. Dissolve 0.1 g in *water* with gentle heating and dilute to 10 ml with *water*. Add 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*, the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*, the solution is red.

Absorbance (2.4.7). Not more than 0.04 at 480 nm, determined in a 2 per cent w/v solution in a mixture of equal volumes of *methanol* and *water*, measure immediately.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 35 volumes of mobile phase B and 65 volumes of mobile phase A.

Test solution. Dissolve 0.1 g of the substance under examination in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a). Dilute 4.0 ml of the test solution to 100.0 ml with a 0.005 per cent w/v solution of *anisaldehyde* in the solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 2.6 g of *sodium octane sulphonate* in 1000 ml of *water*, add 3 ml of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid*,
  B. a mixture of 18 volumes of *acetonitrile* and 82 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
5	65	35
20	20	80
25	20	80

Name	Relative retention time	Correction factor
Dobutamine impurity A[1]	0.3	1.4
Dobutamine impurity B[2]	0.5	1.4
Dobutamine (Retention time: about 12 minutes)	1.0	—
Dobutamine impurity C[3]	1.4	—

[1]dopamine,

[2]4-(4-hydroxyphenyl)butan-2-one,

[3](2*RS*)-*N*-[2-(3,4-dimethoxyphenyl)ethyl]-4-(4-methoxyphenyl) butan-2-amine.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to dobutamine and anisaldehyde is not less than 4.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to dobutamine impurities A, B and C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.25 g in 10 ml of *anhydrous formic acid*. Add 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M perchloric acid* is equivalent to 0.03379 g of $C_{18}H_{24}ClNO_3$.

Storage. Store protected from light.

Dobutamine Injection

Dobutamine Intravenous Infusion

Dobutamine Injection is a sterile solution containing Dobutamine Hydrochloride. It is supplied as a ready-to-use solution or it is prepared by diluting either Sterile Dobutamine

Concentrate or Dobutamine Hydrochloride for Injection with a suitable diluent in accordance with the manufacturer's instructions.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Bacterial endotoxins (2.2.3). Not more than 10 Endotoxin Units per ml of a 1 per cent w/v solution of Dobutamine in water for injections.

Storage. Dobutamine Injection prepared from Sterile Dobutamine Concentrate or from Dobutamine Hydrochloride for Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

When supplied as ready to use solution, the injection complies with the following requirements

Dobutamine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dobutamine, $C_{18}H_{23}NO_3$.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14) as described under test for Related substances for Sterile Dobutamine Concentrate using the following modifications.

Test solution. Dilute a volume of injection with the solvent mixture to obtain a solution of 0.05 per cent w/v of dobutamine.

Assay. Determine by liquid chromatography (2.4.14) as described under Assay for Sterile dobutamine Concentrate with the following modifications.

Test solution. Dilute a volume of injection with the mobile phase to obtain a solution of 0.05 per cent w/v of dobutamine.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of dobutamine.

Sterile Dobutamine Concentrate

Sterile Dobutamine Concentrate is a sterile solution of Dobutamine Hydrochloride in Water for Injections.

Sterile Dobutamine Concentrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dobutamine, $C_{18}H_{23}NO_3$.

The concentrate complies with the requirements for Concentrates for Injections or Injections stated under Parenteral Preparations and with the following requirements.

Description. A colourless or pale yellow solution.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 2.5 to 4.0, determined in the concentrate diluted, if necessary with *water* to obtain a solution containing 1.25 per cent w/v of dobutamine.

Appearance of solution. A solution of concentrate containing 1.25 per cent w/v of dobutamine in *water* is not more opalescent than opalescence solution (OS2) (2.4.1).

Light absorption. The absorbance of a concentrate containing 1.25 per cent w/v of dobutamine in *water* at 400 nm (2.4.7) is not more than 0.2.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Dilute a volume of concentrate to obtain a solution of 0.5 per cent w/v of dobutamine with the solvent mixture.

Reference solution (a). Dilute 4.0 ml of the test solution to 100.0 ml with a 0.005 per cent w/v solution of *anisaldehyde* in the solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 2.6 g of *sodium octanesulphonate* in 1000 ml of *water*, add 3 ml of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid*,
  B. a mixture of 18 volumes of *acetonitrile* and 82 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
5	65	35
20	20	80
25	20	80
28	65	35

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to dobutamine hydrochloride and anisaldehyde is not less than 4.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of concentrate with the mobile phase to obtain a solution containing 0.05 per cent w/v of dobutamine.

Reference solution (a). A 0.06 per cent w/v solution of *dobutamine hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.06 per cent w/v of *dobutamine hydrochloride IPRS* and 0.03 per cent w/v of *4-(4- hydroxyphenyl)butan-2-one* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm) (Such as Supelcosil LC-18-DB),
- mobile phase: a mixture of 14 volumes of *methanol*, 28 volumes of *acetonitrile*, 58 volumes of a solution prepared by dissolving 3.38 g of *sodium octane sulphonate* in 1000 ml of *water* adding 3 ml of *triethylamine*, adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm.
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless, the resolution between the peaks due to dobutamine hydrochloride and 4-(4- hydroxyphenyl)butan-2-one is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{23}NO_3$ in the concentrate.

Labelling. The label states the quantity of active ingredients in terms of the equivalent amount of dobutamine.

Dobutamine Hydrochloride for Injection

Dobutamine Hydrochloride for Injection is a sterile material consisting of Dobutamine Hydrochloride with or without excipients. It is supplied in a sealed container.

Dobutamine Hydrochloride for Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dobutamine, $C_{18}H_{23}NO_3$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 2.5 to 4.5, determined in a solution of injection containing 2.5 per cent w/v of dobutamine in *water*.

Appearance of solution. A solution of injection containing 2.5 per cent w/v of dobutamine in *water* is not more opalescent than opalescence solution (OS2) (2.4.1).

Light absorption. The absorbance of a solution containing 2.5 per cent w/v of dobutamine in *water* at 480 nm (2.4.7) is not more than 0.065.

Related substances. Determine by liquid chromatography (2.4.14) as described under test for Related substances for Sterile Dobutamine Concentrate using the following modifications.

Test solution. Dissolve a quantity of the contents of the sealed container with the solvent mixture to obtain a solution containing 0.5 per cent w/v of dobutamine.

Water (2.3.43). Not more than 2.0 per cent (*NOTE —Use the contents of a single container and add 7 g of salicylic acid and 20 ml of formamide before the determination*).

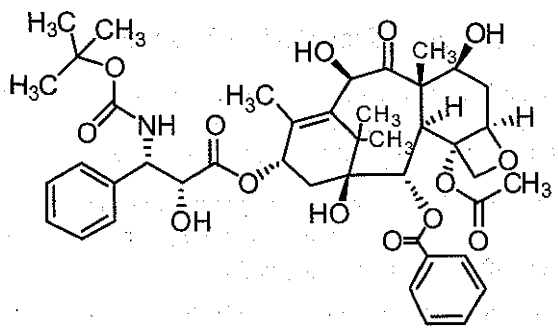
Bacterial endotoxins (2.2.3). Not more than 5.56 Endotoxin Units per mg of dobutamine.

Assay. Determine by liquid chromatography (2.4.14) as described under Assay for Sterile Dobutamine Concentrate using the following modifications.

Test solution. Dissolve sufficient of the mixed contents of 10 containers in the mobile phase to obtain a solution containing 0.05 per cent w/v of Dobutamine.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of dobutamine.

Docetaxel Anhydrous



$C_{43}H_{53}NO_{14}$ Mol. Wt. 807.9

Docetaxel Anhydrous is 1β,7β,10β-trihydroxy-9-oxo-5β,20-epoxytax-11-ene-2α,4α,13α-triyl 4-acetate 2-benzoate 13-{(2*R*,3*S*)-3-[(*tert*-butoxycarbonyl)amino]-2-hydroxy -3-phenylpropanoate}.

Docetaxel Anhydrous contains not less than 97.5 per cent and not more than 102.0 per cent of $C_{43}H_{53}NO_{14}$, Calculated on the anhydrous basis.

Description. A white or almost white, crystalline, hygroscopic powder.

Identification

A. Specific optical rotation (2.4.22). Complies the test.

B. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *docetaxel anhydrous IPRS* or with the reference spectrum of docetaxel anhydrous.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *ethanol* is not more opalescent than reference suspension II (2.4.1) and not more intensely coloured than reference solution BS5 (2.4.1).

Specific optical rotaion (2.4.22). −41.5° to −38.5°, determined in 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.05 volume of *acetic acid*, 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in 2.5 ml of *ethanol* and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dissolve 50 mg of *docetaxel trihydrate IPRS* in 2.5 ml of *ethanol* and dilute to 50.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (c). Dissolve 5 mg of *docetaxel impurity E IPRS* in 2.5 ml of *ethanol* and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- column temperature. 45°,
- mobile phase: A. *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 232 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	72	28
9	72	28
39	28	72
42	72	28

Name	Relative retention time	Correction factor
Docetaxel impurity E[1]	0.2	—
Docetaxel impurity A[2]	0.97	1.6
Docetaxel (Retention time is about 27 minutes)	1.0	—
Docetaxel impurity B[3]	1.08	—
Docetaxel impurity C[4]	1.13	—

[1]10-desacetyl-baccatin III,

[2]2-*O*-desbenzoyl-2-*O*-tiglyldocetaxel,

[3]10-deoxy-10-oxodocetaxel,

[4]7-*epi*-docetaxel.

Inject reference solution (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to docetaxel impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any peak due to docetaxel impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any peak due to docetaxel impurity C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any peak due to docetaxel impurity E is not more than 1.5 times the area of corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent), the area of any other secondary peak is not

more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). Dissolve 1.0 g in 20 ml of a mixture of 15 volumes of *water* and 85 volumes of *dimethylformamide*. 12 ml of the solution complies with the limit test for heavy metals, method D (20 ppm), using 10 ml of *lead standard solution (1 ppm Pb)*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14) as described under test for Related substances with the following modification.

Inject reference solution (a) and the test solution.

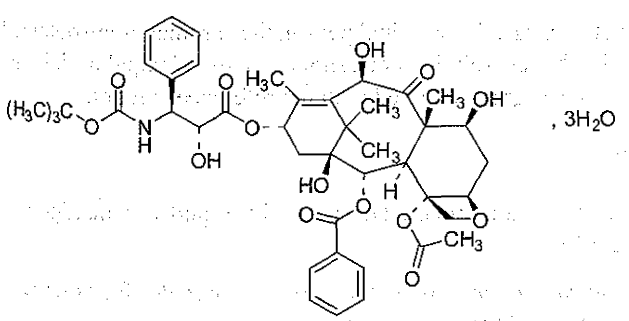
Calculate the content of $C_{43}H_{53}NO_{14}$.

If intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

Bacterial endotoxins (2.2.3). Not more than 0.3 Endotoxin Unit per mg of docetaxel

Storage. Store protected from light and moisture.

Docetaxel Trihydrate



$C_{43}H_{53}NO_{14}, 3H_2O$ Mol. Wt. 861.9

Docetaxel Trihydrate is *N*-debenzoyl-*N*-(*tert*-butoxycarbonyl)-10-deacetyltaxol trihydrate.

Docetaxel Trihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{43}H_{53}NO_{14}$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *docetaxel trihydrate IPRS* or with the reference spectrum of docetaxel trihydrate.

B. In the Assay, the retention time of principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). −41.5° to −38.5°, determined in 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 0.05 volume of *acetic acid*, 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in 2.5 ml of *ethanol* and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dissolve 50 mg of *docetaxel trihydrate IPRS* in 2.5 ml of *ethanol* and dilute to 50.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 45°,
- mobile phase: A. *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 232 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	72	28
9	72	28
39	28	72
42	72	28

Name	Relative retention time	Correction factor
Docetaxel impurity A[1]	0.97	1.6
Docetaxel (Retention time is about 27 minutes)	1.0	---
Docetaxel impurity B[2]	1.08	—
Docetaxel impurity C[3]	1.13	—
Docetaxel impurity D[4]	1.18	—

[1]2-*O*-desbenzoyl-2-*O*-tiglyldocetaxel,

[2]10-deoxy-10-oxodocetaxel,

[3]7-*epi*-docetaxel,

[4]4-*epi*-6-oxodocetaxel.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to docetaxel impurity A is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak due to docetaxel impurity B and docetaxel impurity C is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any peak due to docetaxel impurity D is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g of complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). 5.0 per cent to 7.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{43}H_{53}NO_{14}$.

Docetaxel Trihydarte intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.3 Endotoxin Unit per mg of docetaxel.

Storage. Store protected from light and moisture, at a temperature not exceeding 8°.

Docetaxel Injection

The injection is constituted by dissolving the contents of the sealed container in accordance with the manufacturer's instructions, immediately before use.

The constituted solution complies with the requirements for clarity of solution and particulate matter stated under Parenteral Preparations (Infusions).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Usual strengths. 20 mg; 80 mg; 120 mg.

Docetaxel Concentrate

Docetaxel Concentrate is a sterile solution of Docetaxel in a suitable vehicle.

The concentrate complies with the requirements stated under Parenteral Preparations (Concentrated solutions for injections) and with the following requirements.

Docetaxel Concentrate contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of docetaxel, $C_{43}H_{53}NO_{14}$.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 100 volumes of *acetonitrile*, 0.1 volume of *acetic acid* and 100 volumes of *water*.

Test solution.

For the injection labeled as one-vial formulation — Dilute a portion of the injection to obtain a solution containing 0.02 per cent w/v of docetaxel (anhydrous) in the solvent mixture.

For the injection labeled as two-vial formulation — Dissolve the content of the vial in an amount of alcohol equivalent to

5.0 per cent of the final volume and dilute with the solvent mixture to obtain a solution containing 0.02 per cent w/v of docetaxel (anhydrous) in the solvent mixture.

Reference solution (a). A 0.02 per cent w/v solution of *docetaxel IPRS* prepared by dissolving in *ethanol (95 per cent)* and diluting with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Further dilute 1.0 ml to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 45°,
- sample temperature: 10°,
- mobile phase: A. *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 232 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	72	28
9	72	28
39	28	72
39.1	0	100
49	0	100
49.1	72	28
60	72	28

Name	Relative retention time	Correction factor
10-deacetyl baccatin[1]	0.27	0.67
2-debenzoxyl 2-pentenoyl docetaxel[2]	0.97	—
docetaxel	1.0	—
crotonaldehyde analog[3]	1.05	—
6-oxodocetaxel[4]	1.08	—
4-epidocetaxel[5]	1.13	—
4-epi-6-oxodocetaxel[6]	1.18	—

[1](2a*R*, 4*S*, 4a*S*, 6*R*, 9*S*, 11*S*,12*S*,12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11,12, 12a,12b-dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H* -cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-benzoate,

[2](2a*R*, 4*S*, 4a*S*, 6*R*, 9*S*, 11*S*, 12*S*, 12a*R*,12b*S*)-1,2a,3,4,4a,6,9,10,11, 12,12a,12b-dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H* -cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-[(*E*)-2-methylbut-2-enoate], 9-ester with (2*R* ,3*S*)-*N*-tert-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β, 20-epoxy-1,7β,10β-trihydroxy-9-oxotax-11-ene-2α,4,13α-triyl-4-acetate-13[(2R,3S)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate]-2-[(2E)-2-methylbut-2-enoate]. It is a process impurity for identification only. It is controlled in the API. It is not reported in the formulations and should not be included in the Total impurities,

[3](1*S*, 2*S*, 3*R*, 9*S*, *E*)-3-[(*S*,*E*)-2-acetoxy-1-hydroxy-5-oxopent-3-en-2-yl]-1,5,9-trihydroxy-4,8,11,11-tetramethyl-6-oxobicyclo[5.3.1]undeca-4,7-dien-2-yl benzoate, 9-ester with (2*R*, 3*S*)-N-tert-butoxycarbonyl-3-phenylisoserine,

[4](2a*R*, 4*S*, 4a*S*, 9*S*, 11*S*, 12*S*, 12a*R*, 12b*S*)-1,2a,3,4,4a,6,9,10,11,12, 12a,12b-dodecahydro-4, 9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H* -cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2*R*, 3*S*)-N-tert-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β, 20-epoxy-1,7β,dihydroxy-9,10-dioxotax-11-ene-2α,4,13α-triyl-4-acetate-2-benzoate-13[(2R,3S)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate],

[5](2a*R*, 4*R*, 4a*S*, 6*R*, 9*S*, 11*S*, 12*S*, 12a*R*, 12b*S*)-1,2a,3,4,4a,6,9,10,11,12, 12a,12b-dodecahydro-4,6,9,11,12,12b-hexahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H* -cyclodeca[3,4]benz[1,2-b]oxet-5-one 12b-acetate, 12-benzoate, 9-ester with (2*R*, 3*S*)-N-tert-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β, 20-epoxy-1, 7α, 10β-trihydroxy-9-oxotax-11-ene-2α, 4, 13α-triyl-4-acetate-2-benzoate-13[(2R,3S)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate],

[6](2a*R*, 4*R*, 4a*S*, 9*S*, 11*S*, 12*S*, 12a*R*, 12b*S*)-1,2a,3,4,4a,6,9,10,11,12, 12a,12b-dodecahydro-4, 9,11,12,12b-pentahydroxy-4a,8,13,13-tetramethyl-7,11-methano-5*H* -cyclodeca[3,4]benz[1,2-b]oxet-5,6-dione 12b-acetate, 12-benzoate, 9-ester with (2*R*, 3*S*)-*N*-tert-butoxycarbonyl-3-phenylisoserine. The alternative chemical name is 5β, 20-epoxy-1,7α-dihydroxy-9,10-dioxotax-11-ene-2α,4,13α-triyl-4-acetate-2-benzoate-13[(2R,3S)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-3-phenylpropanoate].

Inject reference solution (a) and (b). The test is not valid unless the signal to noise ratio is not less than 10 for reference solution (b), and relative standard deviation for replicate injections is not more than 1.0 per cent for reference solution (a).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any peak due to 10-deacetyl baccatin is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any peak due to crotonaldehyde analog is not more than 13 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.3 per cent), the area of any peak due to 6-oxodocetaxel is not more than 15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent), the area of any peak due to 4-epidocetaxel is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any peak due to 4-epi-6-oxodocetaxel is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum

of the areas of all the secondary peaks is not more than 35 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.5 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1per cent) and any peak with a relative retention time less than 0.2 or more than 1.3.

Bacterial endotoxins (2.2.3). Not more than 1.94 Endotoxin Units per mg of docetaxel (anhydrous).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances using following modifications.

NOTE—Use the solution within 24 hours when stored at 25°.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

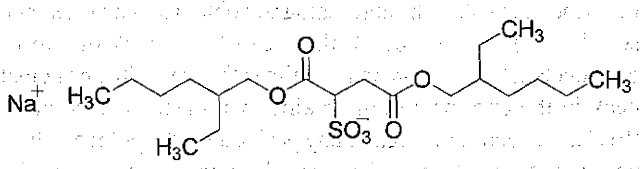
Calculate the content of $C_{43}H_{53}NO_{14}$ in the injection.

Storage. Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store at a temperature not exceeding 25°.

Labelling. The label states (1) The strength in terms of the equivalent amount of Docetaxel anhydrous; (2) Indicate whether it is a one-vial formulation or two-vial formulation (Injection concentrate and diluent), and also label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.

Docusate Sodium

Dioctyl Sodium Sulphosuccinate



$C_{20}H_{37}NaO_7S$ Mol. Wt. 444.6

Docusate Sodium is sodium 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulphonate.

Docusate Sodium contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{20}H_{37}NaO_7S$, calculated on the anhydrous basis.

Category. Emollient laxative or purgative.

Description. White or almost white, waxy masses or flakes, hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *docusate sodium IPRS* or with the reference spectrum of docusate sodium.

B. Ignite 0.75 g in the presence of *dilute sulphuric acid*, until an almost white residue is obtained. Cool and add 5 ml of *water*, filter. 2 ml of the filtrate gives reaction (A) of sodium salts (2.3.1).

Tests

Alkalinity. Dissolve 1.0 g in 100 ml of a mixture of equal volumes of *methanol* and *water*, previously neutralised to *methyl red solution*. Add 0.1 ml of *methyl red solution*. Not more than 0.2 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator to red.

Related non-ionic substances. Determine by gas chromatography (2.3.13).

Internal standard solution. Dissolve 10 mg of *methyl behenate* in 50 ml of *hexane*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 2.0 ml of the internal standard solution and dilute to 5.0 ml with *hexane*. Pass the solution, at a rate of about 1.5 ml per minute, through a column 10 mm in internal diameter, packed with 5 g of *basic aluminium oxide* and previously washed with 25 ml of *hexane*. Elute with 5 ml of *hexane* and discard the eluate. Elute with 20 ml of a mixture of equal volumes of *ether* and *hexane*. Evaporate the eluate to dryness and dissolve the residue in 2.0 ml of *hexane*.

Test solution (b). Prepare as described for test solution (a) but dissolving 0.1 g of the substance under examination in 5.0 ml of *hexane* and using a new column.

Reference solution. Dilute 2.0 ml of the internal standard solution to 5.0 ml with *hexane*.

Chromatographic system

- a glass column 2 m x 2 mm, packed with silanised diatomaceous earth for gas chromatography (150 µm to 180 µm) impregnated with 3 per cent m/m of *polymethylphenylsiloxane*,
- temperature :
  column 230°,
  inlet port and detector at 280°,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of test solution (a), (b) and the reference solution. There is no peak with the same retention time as the internal

standard in the chromatogram obtained with test solution (b). The area of any impurity peak is not more than the area of the peak due to the internal standard (0.4 per cent).

Chlorides (2.3.12). Dissolve 5.0 g in 50 ml of *alcohol (50 per cent v/v)* and add 0.1 ml of *potassium dichromate solution*. Not more than 0.5 ml of *0.1 M silver nitrate* is required to change the colour of the indicator from yellow to orange (350 ppm).

Sodium sulphate. Not more than 2 per cent.

Dissolve 0.25 g in 40 ml of a mixture of 20 volumes of *water* and 80 volumes of *2-propanol*. Adjust to pH between 2.5 and 4.0 using *perchloric acid solution*. Add 0.4 ml of *naphtharson solution* and 0.1 ml of 0.0125 per cent w/v solution of *methylene blue*. Not more than 1.5 ml of *0.025 M barium perchlorate* is required to change the colour of the indicator from yellowish-green to yellowish-pink.

Heavy metals (2.3.13). Dissolve 4.0 g in 20 ml of *ethanol (80 per cent v/v)*. 12 ml of the solution complies with the limit test for heavy metals, Method D (10 ppm), using 10 ml of *lead standard solution (2 ppm Pb)*.

Water (2.3.43). Not more than 3.0 per cent, determined on 0.25 g.

Assay. Dissolve 1.0 g in 25.0 ml of *0.5 M alcoholic potassium hydroxide* and heat on a water-bath under reflux for 45 minutes. Cool, add 0.25 ml of *phenolphthalein solution* and titrate with *0.5 M hydrochloric acid* until the red colour disappears. Carry out a blank titration.

1 ml of *0.5 M hydrochloric acid* is equivalent to 0.1112 g of $C_{20}H_{37}NaO_7S$.

Storage. Store protected from moisture.

Docusate Tablets

Docusate Sodium Tablets

Docusate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of docusate sodium, $C_{20}H_{37}NaO_7S$.

Usual strength. 100 mg.

Identification

Extract the powder of the tablets with solvent hexane, filter, and evaporate the filtrate on a water-bath. Determine by infrared absorption spectrophotometry (2.4.6) on the residue. Compare the spectrum with that obtained with *docusate sodium IPRS* or with the reference spectrum of docusate sodium.

Tests

Disintegration (2.5.1). Not more than 60 minutes.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse 10 tablets with 200 ml of *ethanol (95 per cent)* and 300 ml of *water*, shake by mechanical means for about 90 minutes and dilute to 1000 ml with *water*, filter.

Reference solution (a). Dissolve a weighed quantity of *docusate sodium IPRS* in *ethanol (95 per cent)* and dilute with *water* to obtain a solution having a concentration of 0.1 per cent w/v of Docusate Sodium.

Reference solution (b). Dissolve about 15 mg of *methylparaben* in 100 ml of *water*. To 0.1 ml of the solution, add 10 ml of reference solution (a).

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 50 volumes of *7 mM ammonium acetate* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 40 µl.

The relative retention time with reference to docusate for methylparaben is about 0.74.

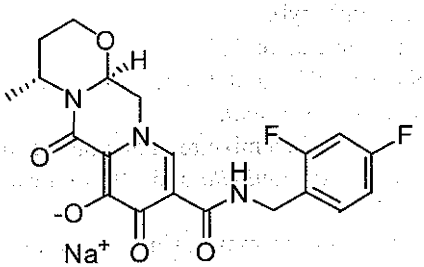
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to methylparaben and docusate is not less than 2.0, the tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 1.8 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{20}H_{37}NaO_7S$ in the tablets.

Storage. Store protected from moisture.

Dolutegravir Sodium



$C_{20}H_{18}F_2N_3NaO_5$ Mol. Wt. 441.4

Dolutegravir Sodium is sodium (4*R*,12a*S*)-9-[(2,4-difluorobenzyl)carbamoyl]-4-methyl-6,8-dioxo-3,4,6,8,12,12a-

hexahydro-2*H*-pyrido[1',2':4,5]pyrazino[2,1-*b*][1,3]oxazin-7-olate.

Dolutegravir Sodium contains not less than 98.0 per cent and not more than 102. 0 per cent of $C_{20}H_{18}F_2N_3NaO_5$, calculated on the anhydrous basis.

Category. Antiretroviral.

Description. A white to light yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dolutegravir sodium IPRS* or with the reference spectrum of dolutegravir sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Ignite 0.1 g of substance under examination and dilute to 2.0 ml with *water*. The solution gives reaction (A) of sodium (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.05 per cent w/v solution of *dolutegravir sodium IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture. Further, dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with phenyl group bonded to porous silica (5 µm) (Such as Kinetex Biphenyl),
- column temperature: 35°,
- mobile phase: A. a 0.1 per cent v/v solution of *orthophosphoric acid*,
  B. a mixture of 50 volumes of *water*, 40 volumes of *acetonitrile* and 10 volumes of *tertiary butanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	50	50
5	50	50
20	40	60
40	40	60
41	50	50
50	50	50

Name	Relative retention time	Correction factor
Hydroxy impurity[1]	0.54	—
Methyl dolutegravir[2]	0.76	1.39
2-fluoro impurity[3]	0.89	—
4-fluoro impurity[4]	0.92	—
Dolutegravir sodium	1.0	—
Isomer-1[5]+ isomer-2[6]	1.05	—

[1]N-(2,4-difluorobenzyl)-9-hydroxy-2-[(2*R*)-4-hydroxybutan-2-yl]-1,8-dioxo-1,8-dihydro-2*H*-pyrido[1,2-a]pyrazine-7-carboxamide,

[2](4*R*,12a*S*)-N-(2,4-difluorobenzyl)-7-methoxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2',:4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide ,or (4*R*,12a*S*)-N-(2,4-difluorobenzyl)-7-methoxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-[1,3]oxazino[3,2-d]pyrido[1,2-a]pyrazine-9-carboxamide,

[3](4*R*,12a*S*)-*N*-(2-fluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide or 2- fluorobenzyl impurity,

[4](4*R*,12a*S*)-N-(4-fluorobenzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide or Desfluoro impurity,

[5]sodium (4*S*,12a*S*)-9-[(2,4-difluorobenzyl) carbamoyl]-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5]pyrazino[2,1-b][1,3]oxazin-7-olate,

[6](4*R*,12a*R*)-*N*-[(2,4-difluorobenzyl) -7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2',:4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to hydroxyl impurity, methyl dolutegravir impurity, 2- fluoro impurity and 4- fluoro impurity peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with the

reference solution (1.0 per cent). Ignore the peak due to isomer 1, isomer 2 and the peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.025 per cent w/v solution of *dolutegravir for system suitability IPRS* (containing 4-fluoro impurity, isomer-1, isomer-2 and enantiomer) in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with Chiralcel (5 µm), (Such as Chiralcel OZ-RH),
- column temperature: 30°,
- mobile phase: a mixture of 94 volumes of *acetonitrile*, 6 volumes of *water* and 0.2 volume of *formic acid*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Name	Relative retention time
Isomer-1	0.82
Enantiomer[1]	0.89
Dolutegravir	1.0
Isomer-2	1.22

[1]Sodium (4*S*,12a*R*)-9-[(2,4-difluorobenzyl) carbamoyl]-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1',2':4,5]pyrazino[2,1-b][1,3]oxazin-7-olate.

Inject the reference solution. The test is not valid unless the resolution between isomer-1 peak and enantiomer peak is not less than 1.2.

Inject the test solution. The area of dolutegravir isomer-1, isomer-2 and enantiomer peak, each of, is not more than 0.15 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *dolutegravir sodium IPRS* in the solvent mixture

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with phenyl group bonded to porous silica (5 µm) (Such as Kinetex Biphenyl),
- column temperature: 50°,
- mobile phase: a mixture of 0.1 volume of *orthophosphoric acid*, 60 volumes of *water*, 20 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{18}F_2N_3NaO_5$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Dolutegravir Tablets

Dolutegravir Sodium Tablets

Dolutegravir Tablets contain dolutegravir sodium equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dolutegravir, $C_{20}H_{19}F_2N_3O_5$.

Usual strength. 50 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of 0.25 per cent w/v of *sodium lauryl sulphate* in a buffer solution prepared by dissolving 1.2 g of *anhydrous sodium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 6.8 with *0.2 M sodium hydroxide solution*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *dolutegravir sodium IPRS* in the solvent mixture and dilute if necessary with the dissolution medium to obtain a solution of about the same concentration as the test solution.

Chromatographic system
- a stainless steel column 7.5 cm × 4.6 mm, packed with octylsilane bonded to porous silica (2.6 µm) (Such as Kinetex C8, 100 Å),
- mobile phase: a mixture of 65 volumes of 0.1 per cent v/v of *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 258 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{19}F_2N_3O_5$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{19}F_2N_3O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Disperse a quantity of powdered tablets containing 100 mg of Dolutegravir in 70 ml of the solvent mixture, with the aid of ultrasound for about 30 minutes with intermittent shaking and dilute to 100.0 ml with the solvent mixture. Centrifuge at 5000 rpm for 10 minutes and use supernatant liquid.

Reference solution (a). A 0.0025 per cent w/v solution each of *dolutegravir isomer-1 IPRS* and *isomer-2 IPRS* in the solvent mixture.

Reference solution (b). Dissolve 20 mg of *dolutegravir sodium IPRS* in 4 ml of reference solution (a) and dilute to 20.0 ml with the solvent mixture.

Reference solution (c). A 0.001 per w/v solution of *dolutegravir sodium IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3µm),
- column temperature: 40°,
- sample temperature: 10°,
- mobile phase: A. a mixture of 95 volumes of *0.01 M potassium dihydrogen orthophosphate*, adjusted to pH 2.8 with *dilute orthophosphoric acid* and 5 volumes of *acetonitrile*,

B. a mixture of 20 volumes of *0.01 M potassium dihydrogen orthophosphate*, adjusted to pH 2.8 with *dilute orthophosphoric acid* and 80 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 255 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
10	90	10
20	80	20
30	65	35
70	75	25
77	30	70
82	20	80
85	20	80
90	90	10
100	90	10

Name	Relative retention time
Hydroxy impurity	0.65
Methyl dolutegravir impurity*	0.80
2-Fluoro impurity*	0.82
Desfluoro (4-fluoro) impurity*	0.86
Dolutegravir	1.00
Dolutegravir isomer-1* and isomer-2*	1.02

*Process impurity included for identification only and not included in the calculation of total degradation products.

Inject reference solution (b) and (c). The test is not valid unless the peak to valley ratio is not less than 1.5 for reference solution (b) and the tailing factor is not more than 2.0 for reference solution (c).

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to hydroxy impurity is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water.*

Test solution. Weigh and transfer 10 intact tablets into 500-ml volumetric flask, add 50 ml of *water* and disperse with the aid of ultrasound for about 30 minutes, add 50 ml of *acetonitrile* and 200 ml of the solvent mixture, sonicate for 45 minutes with occasional shaking and dilute to volume with the solvent mixture. Centrifuge at 5000 rpm for 10 minutes. Dilute 1.0 ml of the supernatant liquid to 25.0 ml with the solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *dolutegravir sodium IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Kinetex C18 100 Å),
- sample temperature: 10°,
- mobile phase: a mixture of 65 volumes of 0.1 per cent v/v of *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 258 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{19}F_2N_3O_5$ in the tablets.

1 mg of dolutegravir sodium is equivalent to 0.950 mg of dolutegravir.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of dolutegravir.

Dolutegravir, Lamivudine and Tenofovir Disoproxil Fumarate Tablets

Dolutegravir Sodium, Lamivudine and Tenofovir Disoproxil Fumarate Tablets

Dolutegravir, Lamivudine and Tenofovir Disoproxil Tablets contain an amount of dolutegravir sodium equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dolutegravir, $C_{20}H_{19}F_2N_3O_5$, lamivudine, $C_8H_{11}N_3O_3S$ and tenofovir disoproxil fumarate, $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$.

Usual strength. Dolutegravir 50 mg, Lamivudine 300 mg and Tenofovir Disoproxil Fumarate 300 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a buffer solution prepared by dissolving 6.9 g of *sodium dihydrogen phosphate,* 0.9 g of *sodium hydroxide* and 2.5 g of *sodium dodecyl sulphate* in 800 ml of *water*, adjusted to pH 6.8 with *2 M sodium hydroxide* and dilute to 1000 ml with *water*,

Speed and time. 60 rpm and 30 minutes, 60 minutes.

Withdraw 10 ml of the medium after 30 minutes, filter (solution A). Add 10 ml of dissolution medium in each dissolution vessel and continue the dissolution for further 30 minutes. After 60 minutes, withdraw again 10 ml of the medium, filter (solution B).

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution (a). Dilute 5.0 ml of solution A to 25.0 ml with solvent mixture (b).

Test solution (b). Dilute 5.0 ml of solution B to 25.0 ml with solvent mixture (b).

Inject the reference solution, test solution (a) and (b).

Calculate the content of $C_8H_{11}N_3O_3S$ in test solution (a) and $C_{19}H_{30}N_5O_{10}P$,$C_4H_4O_4$ and $C_{20}H_{19}F_2N_3O_5$ in test solution (b) in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{20}H_{19}F_2N_3O_5$, $C_8H_{11}N_3O_3S$ and $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$.

Related substances.

For lamivudine and tenofovir disoproxil fumarate —

Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 225 mg of Tenofovir Disoproxil Fumarate in 150 ml of *water*, with the aid of ultrasound for 30 minutes with intermittent shaking and dilute to 250.0 ml with *water*, filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *water.*

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 100.0 ml with *water.*

Reference solution (c). Dissolve 1 mg of *tenofovir disoproxil for system suitability IPRS* (containing tenofovir disoproxil impurity I and H) in 2.0 ml of *water.*

Reference solution (d). A 0.02 per cent w/v solution of fumaric acid in *water.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with endcapped octadecylsilane bonded to porous silica, (5 µm),
- sample temperature: 6°,
- mobile phase: A. a buffer solution prepared by dissolving 9.64 g of *ammonium acetate* in 900 ml of water, adjusted to pH 4.2 with *glacial acetic acid*, dilute to 1000 ml with *water*,

B. *acetonitrile*,

- flow rate: 1ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
2	100	0
17	95	5
47	60	40
62	25	75
63	100	0
75	100	0

Name	Relative retention time	Correction factor
Tenofovir disoproxil impurity M[1]	0.33	—
Tenofovir disoproxil impurity A[2]	0.63	0.79
Tenofovir disoproxil impurity F[3]	0.73	—
Tenofovir disoproxil impurity E[4]	0.76	—
Tenofovir disoproxil impurity B[5]*	0.80 and 0.81	—
Tenofovir disoproxil impurity C[6]*	0.88	—
Tenofovir disoproxil impurity D[7]*	0.90	—
Tenofovir disoproxil impurity L[8]	0.94	0.53
Tenofovir disoproxil impurity K[9]*	0.97	—
Tenofovir disoproxil impurity I[10]	0.98	—
Tenofovir disoproxil (Retention time: about 48 minutes)	1.0	—
Tenofovir disoproxil impurity H[11]*	1.01	—
Tenofovir disoproxil impurity J[12]	1.19	—
Lamivudine impurity E[13]	0.09	0.61
Lamivudine impurity F[14]	0.11	0.48
Lamivudine impurity A[15]*	0.15 and 0.17	—
Lamivudine impurity G[16]	0.20	—
Lamivudine impurity H[17]	0.21	—
Lamivudine impurity B[18]*	0.38	—
Lamivudine	0.39	—
Lamivudine impurity J[19]	0.45	—
Lamivudine impurity C[20]*	0.54	—

*Process impurity that is included in the table for identification purposes only. It is controlled in the drug substance and is not to be reported or included in the total degradation products for the drug product,

[1]9-[(R)-2-(Phosphonomethoxy)propyl]adenine (synthesis-related impurity, degradation 260 product),

[2](1-methylethyl) (8*R*)-9-(6-amino-9*H*-purin-9-yl)-5-hydroxy-8-methyl-5-oxo-2,4,7-trioxa-5-λ[5]-phosphanonanoate (tenofovir monosoproxil),

[3]bis(1-methylethyl) 9,9'-[methylenebis(imino-9*H*-purine-6,9-diyl)]bis[(8R)-5-hydroxy-8-methyl-5-oxo-2,4,7-trioxa-5-λ[5]-phosphanonanoate] (tenofovir monosoproxil dimer),

[4](1-methylethyl) (8*R*)-5-hydroxy-8-methyl-9-(6-{[(1-methylethoxy)carbonyl]amino}-9*H*-purin-9-yl)-5-oxo-2,4,7-trioxa-5-λ[5]-phosphanonanoate,

[5](1-methylethyl) (5*RS*,8*R*)-9-(6-amino-9*H*-purin-9-yl)-5-methoxy-8-methyl-5-oxo-2,4,7-trioxa-5-λ[5]-phosphanonanoate,

[6]methyl (1-methylethyl) (5*RS*)-5-{[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl}-5-oxo-2,4,6,8-tetraoxa-5-λ[5]-phosphanonanedioate,

[7](1-methylethyl) (5*RS*,8*R*)-9-(6-amino-9*H*-purin-9-yl)-8-methyl-5-(1-methylethoxy)-5-oxo-2,4,7-trioxa-5-λ[5]-phosphanonanoate,

[8]ethyl 1-methylethyl (5*RS*)-5-{[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl}-5-oxo-2,4,6,8-tetraoxa-5- λ[5]-phosphanonanedioate,

[9](1-methylethyl) (5*RS*)-5-{[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl}-10-methyl-5,9-dioxo-2,4,6,8-tetraoxa-10-aza-5-λ[5]-phosphaundecanoate,

[10]bis(1-methylethyl) 5-{[(1*R*)-2-(6-{[({9-[(2*R*)-5-hydroxy-2,11-dimethyl-5,9-dioxo-3,6,8,10-tetraoxa-5-λ[5]-phosphadodecyl]-9*H*-purin-6-yl}amino)methyl]amino}-9*H*-purin-9-yl)-1-methylethoxy]methyl}-5-oxo-2,4,6,8-tetraoxa-5-λ[5]-phosphanonanedioate (tenofovir di- and monosoproxil heterodimer),

[11]1-methylethyl propyl (5*RS*)-5-{[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl}-5-oxo-2,4,6,8-tetraoxa-5-λ[5]-phosphanonanedioate,

[12]tetrakis(1-methylethyl) 5,5'-(methylenebis{imino-9*H*-purine-6,9-diyl[(2*R*)-propane-1,2-diyl]oxymethylene})bis[5-oxo-2,4,6,8-tetraoxa-5-λ[5]-phosphanonanedioate] (tenofovir disoproxil dimer),

[13]4-aminopyrimidin-2(1*H*)-one (cytosine),

[14] pyrimidine-2,4(1*H*,3*H*)-dione (uracil),

[15](2*RS*,5*SR*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid,

[16]4-amino-1-[(2*R*,3*S*,5*S*)-2-(hydroxymethyl)-3-oxo-1,3λ[4]-oxathiolan-5-yl]pyrimidin-2(1*H*)-one,

[17]4-amino-1-[(2*R*,3*R*,5*S*)-2-(hydroxymethyl)-3-oxo-1,3λ[4]-oxathiolan-5-yl]pyrimidin-2(1*H*)-one,

[18]4-amino-1-[(2*RS*,5*RS*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one,

[19]1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidine-2,4(1*H*,3*H*)-dione,

[20]2-hydroxybenzoic acid (salicylic acid).

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to tenofovir disoproxil impurity I and tenofovir disoproxil is not less than 1.5 and the resolution between the peaks due to tenofovir disoproxil and tenofovir disoproxil impurity H is not less than 1.2.

Inject reference solution (d) for identification of peak due to fumaric acid.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak corresponding to tenofovir impurity A is not more than 3 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with reference solution (a) (3.0 per cent), the area of any secondary peak corresponding to each of tenofovir impurity F, impurity I and impurity J is not more than 0.75 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with the reference solution (a) (0.75 per cent), the area of any secondary peak corresponding to each of tenofovir impurity L, impurity E is not more than twice the area of the peak due to tenofovir disoproxil in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any secondary peak corresponding to, each of lamivudine impurity E, impurity F, impurity G, impurity H or impurity J, is not more than twice the area of the peak due to lamivudine in the chromatogram obtained with reference solution (b) (0.2 per cent), the sum of the areas of secondary peaks corresponding to lamivudine impurity G, impurity H, impurity J, impurity E and impurity F is not more than the area of the peak due to lamivudine peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the peak due to lamivudine in the chromatogram obtained with reference solution (b) (0.05 per cent) and the sum of the areas of secondary peaks corresponding to tenofovir impurity F, impurity E, impurity I, impurity J and impurity L is not more than the area of the peak due to tenofovir disoproxil peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore the peak due to fumaric acid and any peak with an area less than 0.5 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with reference solution (b) (0.05 per cent). The sum of the lamivudine and tenofovir disoproxil related impurities is not more than 5.0 per cent.

For dolutegravir —

Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light, using low-actinic glassware.

Solvent mixture. 60 volumes of *water* and 40 volumes of *acetonitrile*.

Test solution. Disperse a quantity of powdered tablets containing 87.5 mg of Dolutegravir in 180 ml of the solvent mixture, with the aid of ultrasound for 5 minutes and dilute to 250.0 ml with the solvent mixture, filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *water*. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (b). Dissolve 0.5 mg of *dolutegravir sodium for system suitability IPRS* (containing dolutegravir sodium and dolutegravir impurity E) in 1.0 ml of the solvent mixture.

Reference solution (c). Dissolve 1 mg of *dolutegravir sodium for peak identification IPRS* (containing dolutegravir sodium and dolutegravir impurity A, B and D) in 1.0 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica, modified with pentafluorophenyl (5µm) (Such as ACE 5 C18-PFP),
- column temperature: 45°,
- mobile phase: A. a buffer solution prepared by dissolving 0.186 g of *disodium edetate* in 900 ml of *water*, adjusted to pH 2.0 with *orthophosphoric acid*, dilute to 1000 ml with *water*,

  B. 90 volumes of *methanol* and 10 volumes of *tetrahydrofuran*.
- flow rate: 1 ml per minute,
- spectrophotometer set at 320 nm,
- injection volume: 30 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
2	60	40
32	50	50
37	50	50
42	30	70
52	30	70
53	60	40
60	60	40

Name	Relative retention time
Dolutegravir impurity C[1]	0.67
Dolutegravir impurity F[2]	0.70
Dolutegravir impurity D[3]	0.77
Dolutegravir impurity E[4]	0.89
Dolutegravir (Retention time: about 27 minutes)	1.0

[1](4*R*,12a*S*)-*N*-[(phenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a- hexahydro-2*H*-pyrido[1', 2':4,5]pyrazino-[2,1-b][1,3] oxazine-9-carboxamide, Desfluoro dolutegravir (synthesis-related impurity),

[2](4*R*,12a*S*)-*N*-[(2,6-difluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo- 3,4,6,8,12,12a-hexahydro-2*H*-pyrido[1', 2':4,5]pyrazino-[2,1-b][1,3] oxazine-9- carboxamide, 2,6-Difluoro dolutegravir (synthesis-related impurity),

[3](4*R*,12α*S*)-*N*-[(2-fluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12α- hexahydro-2*H*-pyrido[1', 2':4,5]pyrazino-[2,1-b][1,3] oxazine-9-carboxamide, 2-Fluoro dolutegravir (synthesis-related impurity),

[4](4*R*,12α*S*)-*N*-[(4-fluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12α- hexahydro-2*H*-pyrido[1', 2':4,5]pyrazino-[2,1-b][1,3] oxazine-9-carboxamide, 4-Fluoro dolutegravir (synthesis-related impurity).

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to impurity E and dolutegravir is not less than 3.0.

Inject reference solution (c) for identification of dolutegravir impurity A, impurity B and impurity D.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to each of impurity C, impurity D, impurity E or impurity F is not more than the area of peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light using low-actinic glassware.

Solvent mixture (a). 60 volumes of *water* and 40 volumes of *acetonitrile.*

Solvent mixture (b). 90 volumes of phosphate buffer solution prepared by dissolving 12.3 g of *sodium dihydrogen phosphate* in 900 ml of *water,* adjusted to pH 3.0 with *orthophosphoric acid,* dilute to 1000 ml with *water* and 10 volumes of *acetonitrile.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.34 g of Lamivudine in 400 ml of solvent mixture (a), with the aid of ultrasound for 10 minutes with intermittent shaking and dilute to 500.0 ml with solvent mixture (a), filter. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture (b).

Reference solution. A solution containing 0.0112 per cent w/v of *dolutegravir sodium IPRS*, 0.068 per cent w/v each of *lamivudine IPRS* and *tenofovir disoproxil fumarate IPRS* in solvent mixture (a). Dilute 5.0 ml of the solution to 50.0 ml with solvent mixture (b).

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with endcapped octylsilane bonded to porous silica, (5 µm),
- mobile phase: A. 0.0186 per cent w/v solution of *disodium edetate* in a mixture of 1 volume of *trifluroacetic acid* and 1000 volumes of *water*,
  B. *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 25 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
10	50	50
12	50	50
12.5	98	2
18	98	2

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent of each components.

Inject the reference solution and the test solution.

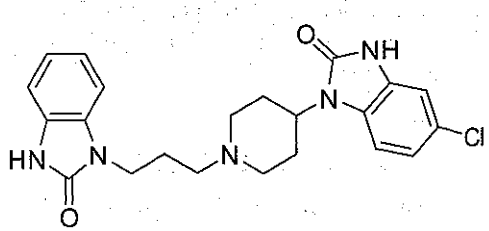
Calculate the content of $C_{20}H_{19}F_2N_3O_5$, $C_8H_{11}N_3O_3S$ and $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$ in the tablets.

1 mg of dolutegravir sodium is equivalent to 0.950 mg of dolutegravir.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the quantity of dolutegravir sodium in terms of the equivalent amount of dolutegravir, and lamivudine and tenofovir disoproxil fumarate.

Domperidone



$C_{22}H_{24}ClN_5O_2$ Mol. Wt. 425.9

Domperidone is 5-chloro-1-[1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-ylpropyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Domperidone contains not than 99.0 per cent and more than 101.0 per cent of $C_{22}H_{24}ClN_5O_2$, calculated on the dried basis.

Category. Antiemetic.

Description. A white or almost white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *domperidone IPRS* or with the reference spectrum of domperidone.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *dimethylformamide* is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 0.1 g of the substance under examination in *dimethylformamide* and dilute to 10.0 ml with the same solvent.

Reference solution (a). A solution containing 0.01 per cent w/v of *domperidone IPRS* and 0.015 per cent w/v of *droperidol IPRS* in *dimethylformamide.*

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *dimethylformamide.* Dilute 5.0 ml of the solution 20.0 ml with *dimethylformamide.*

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with base-deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. 0.5 per cent w/v solution of *ammonium acetate*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
10	0	100
12	0	100
14	70	30

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to domperidone and droperidol is not less than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

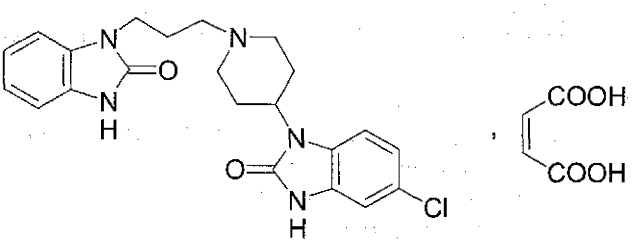
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.3 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid* and 7 volumes of *methyl ethyl ketone.* Titrate with *0.1 M perchloric acid* until the colour changes from orange-yellow to green using 0.2 ml of *naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric* acid is equivalent to 0.04259 g of $C_{22}H_{24}ClN_5O_2$.

Storage. Store protected from light.

Domperidone Maleate



$C_{22}H_{24}ClN_5O_2,C_4H_4O_4$ Mol. Wt. 542.0

Domperidone Maleate is 5-chloro-1-[1-[3-(2,3-dihydro-2-oxo-1*H*-benzimidazol-1-yl)propyl]-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one maleate.

Domperidone Maleate contains not less than 99.0 per cent and not more than 101.0 per cent of domperidone maleate, $C_{22}H_{24}ClN_5O_2,C_4H_4O_4$, calculated on the dried basis.

Category. Antiemetic.

Description. A white or almost white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *domperidone maleate IPRS* or with the reference spectrum of domperidone maleate. If the spectra obtained show differences, dissolve the substance under examination and the reference substance separately in the minimum volume of *2-propanol*, evaporate to dryness on a water-bath and record new spectra using the residues.

Tests

Appearance of solution. Dissolve 0.2 g in *dimethylformamide* and dilute to 20.0 ml with the same solvent. The solution is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE - Prepare the solutions immediately before use.

Test solution. Dissolve 0.1 g of the substance under examination in *dimethylformamide* and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 10 mg of *domperidone maleate IPRS* and 15 mg of *droperidol IPRS* in *dimethylformamide* and dilute to 100 ml with the same solvent.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with *dimethylformamide*. Dilute 5 ml of the solution to 20 ml with *dimethylformamide*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with base-deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. 0.5 per cent w/v solution of *ammonium acetate*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
10	0	100
12	0	100
14	70	30

Equilibrate the column for at least 30 minutes with *methanol* and then equilibrate with the initial mobile phase.

Inject reference solution (a). The retention times are: domperidone maleate, about 6.5 minutes and droperidol, about 7 minutes. The test is not valid unless the resolution between the peaks due to domperidone maleate and droperidol is at least 2.0. If necessary adjust the concentration of *methanol* in the mobile phase or adjust the time programme for the linear gradient.

Inject dimethylformamide as a blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak in the chromatogram obtained with the blank run, any peak due to maleic acid at the beginning of the chromatogram and any peak with an area less than 0.2 times that of the principal peak in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13.). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb)*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° to 105°.

Assay. Dissolve 0.4 g in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid* using 0.2 ml of *naphtholbenzein solution* as indicator, until the colour changes from orange-yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0542 g of $C_{26}H_{28}ClN_5O_6$.

Storage. Store protected from light.

Domperidone Suspension

Domperidone Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of domperidone, $C_{22}H_{24}ClN_5O_2$.

Usual strength. 1 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *dimethylformamide*.

Test solution. Disperse a quantity of the suspension containing 5 mg of Domperidone with 5 ml of *water* and 50 ml of the solvent mixture with the aid of ultrasound for 10 minutes with intermittent shaking. Cool and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *domperidone IPRS* in the solvent mixture. Dilute 5.0 ml of the solution to 10.0 ml with *water* and dilute to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *methanol* and 45 volumes of 0.5 per cent w/v of *ammonium acetate solution*, adjusted to pH 6.15 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of $C_{22}H_{24}ClN_5O_2$.

Domperidone Tablets

Domperidone Maleate Tablets

Domperidone Tablets contain Domperidone Maleate equivalent to not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of domperidone, $C_{22}H_{24}ClN_5O_2$.

Usual strength. 10 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254* or using a precoated plate (such as Merck silica gel 60 F254 plates).

Mobile phase. A mixture of 5 volumes of a solution prepared by dissolving 1.36 g of *sodium acetate* in 50 ml of *water*, adjusted to pH 4.7 with *dilute acetic acid* and adding sufficient *water* to produce 100 ml, 18 volumes of *methanol*, 23 volumes of *dichloromethane* and 54 volumes of *ethyl acetate*.

Test solution. Shake a quantity of the powdered tablets containing 10 mg of domperidone with 10 ml of a mixture of equal volumes of *dichloromethane* and *methanol* and filter through a glass microfibre filter (such as Whatman GF/C).

Reference solution. A 0.127 per cent w/v solution of *domperidone maleate IPRS* in a mixture of equal volumes of *dichloromethane* and *methanol*.

Apply to the plate 10 µl of each solution. After development, allow the plate to dry in air and examine under ultraviolet light at 254 nm. Spray the plate with *potassium iodobismuthate solution* and examine again. With each method of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, diluted with the dissolution medium if necessary, at 286 nm (2.4.7), using the dissolution medium as the blank. Calculate the content of $C_{22}H_{24}ClN_5O_2$ in the medium from the absorbance obtained from a solution of known concentration *domperidone maleate IPRS*.

Q. Not less than 70 per cent of the stated amount of $C_{22}H_{24}ClN_5O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the following solutions immediately before use.

Test solution. To a quantity of the powdered tablets containing 50 mg of domperidone add 10 ml of a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol*, mix with the aid of ultrasound for 20 minutes and filter through a glass microfibre filter (such as Whatman GF/C).

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol*. Dilute 1.0 ml of the resulting solution to 2.0 ml with a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol*.

Reference solution (b). A solution containing 0.01 per cent w/v of *domperidone maleate IPRS* and 0.015 per cent w/v of *droperidol IPRS* in a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with base-deactivated, end-capped octadecylsilyl silica gel (3 µm) (Such as Hypersil BDS),
- mobile phase: A. *methanol*,
  B. a 0.5 per cent w/v solution of *ammonium acetate*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,

– spectrophotometer set at 280 nm,
– injection volume: 10 µl.

Time (in min.)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	30	70
10	100	0
12	100	0
14	30	70

Equilibrate the column for at least 30 minutes with *methanol* and equilibrate with the initial mobile phase for at least 5 minutes.

Inject a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol* as a blank, the test solution and reference solution (a) and (b). The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the two principal peaks is at least 2. If necessary adjust the concentration of *methanol* in the mobile phase or adjust the time programme for the linear gradient.

In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent) and the sum of the areas of any secondary peaks is not greater than twice the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.5 per cent). Ignore any peak in the chromatogram obtained with the blank solution and any peak with an area less than 0.2 times the area of the peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay using the following solution as the test solution.

Test solution. Disperse 1 tablet in a mixture of equal volumes of *0.01 M hydrochloric acid* and *methanol* with the aid of ultrasound to obtain a solution containing 0.01 per cent w/v of domperidone, filter.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *0.01 M hydrochloric acid* and *methanol*.

Test solution. Disperse 10 intact tablets in the solvent mixture to produce a solution containing 0.02 per cent w/v of domperidone, mix with the aid of ultrasound for 30 minutes and filter through a glass microfiber filter (such as Whatman GF/C). Dilute 50.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A 0.0127 per cent w/v solution of *domperidone maleate IPRS* in the solvent mixture.

Use the chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

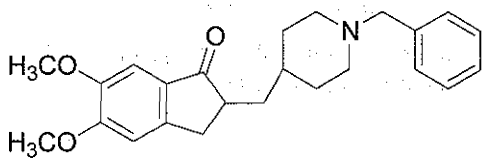
Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}ClN_5O_2$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of domperidone.

Donepezil Hydrochloride



$C_{24}H_{29}NO_3,HCl$ Mol. Wt. 416.0

$C_{24}H_{29}NO_3,HCl,H_2O$ Mol. Wt. 434.0

Donepezil Hydrochloride is (*RS*)-2-[(1-benzyl-4-piperidyl) methyl]-5,6-dimethoxy-1-indanone hydrochloride;

(2RS)-2-[(1-Benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one hydrochloride monohydrate.

Donepezil Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{24}H_{29}NO_3,HCl$, calculated on the anhydrous basis.

Category. Antialzheimer.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *donepezil hydrochloride IPRS* or with the reference spectrum of donepezil hydrochloride.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows absorption maximum at about 230 nm, 268 nm and 313 nm.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *donepezil hydrochloride IPRS* in mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 600 volumes of *water*, 400 volumes of *methanol* and 1 volume of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid* and filter,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 20 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Heavy metals (2.3.13). 1 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.4 per cent for anhydrous form and not more than 7.0 per cent for monohydrate form, determined on 1.0 g.

Assay. Dissolve 0.4 g in a mixture of 40 ml of *glacial acetic acid* and 10 ml of 5 per cent w/v solution of *mercuric acetate* in *glacial acetic acid*. Titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0416 g of $C_{24}H_{29}NO_3,HCl$.

Storage. Store protected from moisture.

Donepezil Tablets

Donepezil Hydrochloride Tablets

Donepezil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of donepezil hydrochloride, $C_{24}H_{29}NO_3,HCl$.

Usual strengths. 5 mg; 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *donepezil hydrochloride IPRS* in the dissolution medium, dilute to obtain a solution having a known concentration similar to expected concentration of test solution.

Chromatographic system as described under Assay using 50 µl injection volume:

Inject the reference solution and the test solution.

Q. Not less than 75 per cent of the stated amount of $C_{24}H_{29}NO_3,HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of *water*.

Test solution. Weigh a quantity of powdered tablets containing about 50 mg of Donepezil Hydrochloride, add 25 ml of solvent mixture, sonicate for 15 minutes and make up the volume to 50.0 ml with solvent mixture and filter.

Reference solution (a). A 0.1 per cent w/v solution of *donepezil hydrochloride IPRS* in solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed octadecylsilane bonded to porous silica (5 µm),
- column temperature. 50°,
- mobile phase: A. a buffer solution pH 6.5 prepared by adding 1 ml of *orthophosphoric acid* in 1000 ml of *water*, adjusted to pH 6.5 with *triethylamine* and filter,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 286 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent w/v)
0	75	25
10	40	60
40	40	60
41	75	25
50	75	25

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 20000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.5 times the area of the peak in the chromatogram obtained with reference solution (b) (1.5 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in the mobile phase with the aid of the ultrasound and dilute to 50.0 ml with the mobile phase. Dilute a suitable volume with the mobile phase to obtain a concentration similar to the reference solution.

Inject the reference solution the test solution.

Calculate the content of $C_{24}H_{29}NO_3$,HCl in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 10 mg of Donezepil Hydrochloride in 70 ml of the mobile phase with the aid of ultrasound for 10 minutes with intermittent shaking and dilute to 100.0 ml with the mobile phase, filter.

Reference solution. A 0.01 per cent w/v solution of *donepezil hydrochloride IPRS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 60 volumes of buffer pH 2.2 prepared by dissolving about 6.8 g of *potassium dihydrogen phosphate* in 1000 ml of *water*. Add 5 ml of *triethylamine* and adjusted to pH 2.2 with *ortho-phosphoric acid*, filter and 40 volumes of *methanol*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume: 20 µl.

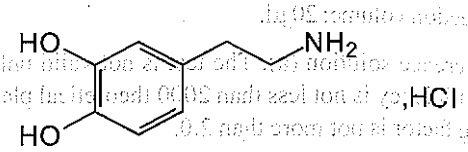
Inject the reference solution. The test is not valid unless the column efficiency is not less than 7000 theoretical plates. The tailing factor is not more than 1.5. The relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution

Calculate the content of $C_{24}H_{29}NO_3$,HCl in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 25°.

Dopamine Hydrochloride



$C_8H_{11}NO_2$,HCl Mol.Wt. 189.6

Dopamine Hydrochloride is 4-(2-aminoethyl)benzene-1,2-diol hydrochloride.

Dopamine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_{11}NO_2$,HCl, calculated on the dried basis.

Category. Vasopressor.

Description. A white or almost white, crystalline powder.

Identification

Tests A, C and D may be omitted if tests B and E are carried out. Test B may be omitted if tests A, C, D and E are carried out.

A. When examined in the range 230 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M hydrochloric acid*, shows absorption maximum at 280 nm and specific absorbance at the absorption maximum is 136 to 150.

B. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dopamine hydrochloride IPRS* or with the reference spectrum of dopamine hydrochloride.

C. Dissolve 5 mg in a mixture of 5 ml of *1 M hydrochloric acid* and 5 ml of *water*. Add 0.1 ml of *sodium nitrite solution* containing 10 per cent w/v solution of *ammonium molybdate*. A yellow colour develops which becomes red on the addition of *strong sodium hydroxide solution*.

D. Dissolve 2 mg in 2 ml of *water* and add 0.2 ml of *ferric chloride solution*. A green colour develops which changes to bluish-violet on the addition of 0.1 g of *hexamethylene-tetramine*.

E. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 4.0 per cent w/v solution in *water* is clear (2.4.1) and not more intensely coloured than reference solution BS6 or YS6 (2.4.1).

Acidity or alkalinity. Dissolve 0.5 g in 10.0 ml of *carbon dioxide-free water*. Add 0.1 ml of *methyl red solution* and 0.75 ml of *0.01 M sodium hydroxide*, the solution becomes yellow. Add 1.5 ml of *0.01 M hydrochloric acid*, the solution becomes red.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Buffer solution. Dissolve 21 g of *citric acid* in 200 ml of *1 M sodium hydroxide* and dilute to 1000 ml with *water*. To 600 ml of the solution, add 400 ml of *0.1 M hydrochloric acid*.

Test solution. Dissolve 50 mg of the substance under examination in mobile phase A and dilute to 25.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Further dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution (b). A solution containing 0.0024 per cent w/v each of dopamine impurity B (*3-O-methyldopamine hydrochloride*) and dopamine impurity A (*4-O-methyldopamine hydrochloride*) in mobile phase A.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with endcapped octadecylsilane bonded to porous silica (4 µm),
- mobile phase: A. dissolve 1.08 g of *sodium octanesulphonate* in 880 ml of the buffer solution, 50 ml of *methanol* and 70 ml of *acetonitrile*,
  B. dissolve 1.08 g of *sodium octanesulphonate* in 700 ml of the buffer solution and add 100 ml of *methanol* and 200 ml of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
5	90	10
20	40	60
25	40	60

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to dopamine impurity B and dopamine impurity A is not less than 5.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. *To avoid overheating, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached.*

Dissolve 0.15 g in 10 ml of *anhydrous formic acid*, add 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01896 g of $C_8H_{12}ClNO_2$.

Storage. Store protected from light and in moisture free container, under nitrogen.

Dopamine Injection

Dopamine Hydrochloride Injection

Dopamine Injection is a sterile solution containing Dopamine Hydrochloride. It is supplied as a ready-to-use solution or it is prepared by diluting either Sterile Dopamine Concentrate or Dopamine Hydrochloride for Injection with a suitable diluent in accordance with the manufacturer's instructions.

Dopamine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dopamine hydrochloride, $C_8H_{11}NO_2,HCl$.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Description. A colourless liquid.

Identification

A. Saturate a volume containing 0.1 g of Dopamine Hydrochloride with *sodium chloride* and extract with three 20-ml quantities of *butan-1-ol*. Filter the combined extracts through anhydrous sodium sulphate and evaporate the filtrate

to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dopamine hydrochloride IPRS* treated in the same manner or with the reference spectrum of dopamine hydrochloride.

B. To a volume containing 10 mg of Dopamine Hydrochloride, add 0.1 ml of a 10.5 per cent w/v solution of *iron(III) chloride hexahydrate*. An intense green colour is produced.

Tests

pH (2.4.24). 3.0 to 4.5

5-Hydroxymethylfurfural. Determine by liquid chromatography (2.4.14).

Test solution. Use the injection.

Reference solution. A 0.0025 per cent w/v solution of *5-hydroxymethylfurfural* in *water*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: *0.05M disodium hydrogen orthophosphate*, adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 284 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 5-hydroxymethylfurfural is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent, determined with reference to the content of glucose).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection to obtain a solution containing 0.032 per cent w/v of Dopamine Hydrochloride with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution and 1.0 ml of a solution containing 0.03 per cent w/v each of *4-ethylcatechol* and *3,4-dimethoxyphenethylamine* to 50.0 ml with the mobile phase.

Use chromatographic system as described under Assay.

The retention time of dopamine is about 5 minutes, of 4-ethylcatechol is about 3 minutes and of 3,4-dimethoxyphenethylamine, is about 12 minutes.

Inject the reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent). The area of not more than one such peak is greater than the area of the principal peak in the chromatogram obtained with the reference solution (a) (2.0 per cent).

Bacterial endotoxins (2.2.3). Not more than 26.67 Endotoxin Units per ml of a 0.16 per cent w/v solution of Dopamine Hydrochloride in water for injections.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the injection with the mobile phase to produce a solution expected to contain 0.0032 per cent w/v of Dopamine Hydrochloride.

Reference solution. A 0.0032 per cent w/v solution of *dopamine hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 2 volumes of *0.1M disodium edetate*, 10 volumes of *glacial acetic acid*, 300 volumes of *acetonitrile* and 700 volumes of *0.005M sodium dodecyl sulphate*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{11}NO_2$,HCl in the injection.

Sterile Dopamine Concentrate

Sterile Dopamine Concentrate is a sterile solution of Dopamine Hydrochloride in Water for Injections.

The concentrate complies with the requirements for Concentrates for Injections or Injections stated under Parenteral Preparations and with the following requirements.

Sterile Dopamine Concentrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dopamine hydrochloride, $C_8H_{11}NO_2$,HCl.

Description. A colourless or pale yellow solution.

Identification

A. Extract a volume containing 0.1 g of Dopamine Hydrochloride with 10 ml of *butan-1-ol*. Filter the extract through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dopamine hydrochloride IPRS* treated in the

same manner or with the reference spectrum of dopamine hydrochloride.

B. To a volume containing 10 mg of Dopamine Hydrochloride, add 0.05 ml of a 10.5 per cent w/v solution of *iron(III) chloride hexahydrate*. An intense green colour is produced.

Tests

pH (2.4.24). 2.5 to 5.5.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 2 volumes of *anhydrous formic acid*, 7 volumes of *water*, 36 volumes of *methanol* and 52 volumes of *chloroform*.

Test solution. Dilute a volume of the concentrate containing 0.15 g of Dopamine Hydrochloride to 5 ml with *methanol*.

Reference solution (a). A 0.0075 per cent w/v solution of *4-O-methyldopamine hydrochloride* in *methanol*.

Reference solution (b). A solution containing 0.0075 per cent w/v each of *3-O-methyldopamine hydrochloride* and *4-O-methyldopamine hydrochloride* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air for 2 hours. Spray evenly and abundantly with a mixture, prepared immediately before use, of equal volumes of *iron (III) chloride solution* and *potassium hexacyanoferrate(III) solution*. Any secondary spot with a R_f value higher than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Bacterial endotoxins (2.2.3). Not more than 26.67 Endotoxin Units per ml of a 0.16 per cent w/v solution of Dopamine Hydrochloride in water for injections.

Assay. Carry out the method described under requirements for the ready-to-use solution.

Storage. Sterile Dopamine Concentrate should be protected from light.

Dopamine Hydrochloride for Injection

Dopamine Hydrochloride for Injection is a sterile material consisting of Dopamine Hydrochloride with or without excipients. It is supplied in a sealed container.

Dopamine Hydrochloride for Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dopamine hydrochloride, $C_8H_{11}NO_2$,HCl.

The contents of the sealed container comply with the requirements for Powders for Injections or Injections stated under Parenteral Preparations and with the following requirements.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dopamine hydrochloride IPRS* or with the reference spectrum of dopamine hydrochloride.

B. Dissolve 10 mg of the substance under examination in 2 ml of *water* and add 0.05 ml of *iron (III) chloride solution*. An intense green colour is produced.

Tests

pH (2.4.24). 2.5 to 5.5 determined in 4.0 per cent w/v solution in a 1.0 per cent w/v solution of *sodium metabisulphite*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 2 volumes of *anhydrous formic acid*, 7 volumes of *water*, 36 volumes of *methanol* and 52 volumes of *chloroform*.

Test solution. Dissolve a quantity of the contents of the sealed container to produce a solution containing 3.0 per cent w/v of dopamine hydrochloride in *methanol*.

Reference solution (a). A 0.0075 per cent w/v solution of *4-O-methyldopamine hydrochloride* in *methanol*.

Reference solution (b). A solution containing 0.0075 per cent w/v each of *3-O-methyldopamine hydrochloride* and *4-O-methyldopamine hydrochloride* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air for 2 hours. Spray evenly and abundantly with a mixture, prepared immediately before use, of equal volumes of *iron(III) chloride solution* and *potassium hexacyanoferrate(III) solution*. Any secondary spot with a R_f value higher than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Bacterial endotoxins (2.2.3). Not more than 26.67 Endotoxin Units per ml of a 0.16 per cent w/v solution of Dopamine hydrochloride in water for injections.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve sufficient quantity of the mixed contents of the 10 containers in the mobile phase to produce a solution containing 0.0032 per cent w/v of Dopamine Hydrochloride

Reference solution. A 0.0032 per cent w/v solution of *dopamine hydrochloride IPRS* in the mobile phase

Use the chromatographic procedure as described under Assay for the ready-to-use solution.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{11}NO_2,HCl$ in a container of average content weight.

Dopamine Hydrochloride and Dextrose Injection

Dopamine Hydrochloride and Dextrose Injection is a sterile solution of Dopamine Hydrochloride and Dextrose in Water for Injection.

Dopamine Hydrochloride and Dextrose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of dopamine hydrochloride, $C_8H_{11}NO_2,HCl$ and dextrose, $C_6H_{12}O_6, H_2O$.

NOTE— Do not use the injection if it is darker than slightly yellow or discoloured in any other way.

Usual strengths. Dopamine Hydrochloride, 80 mg and Dextrose, 5 g per, 100 ml; Dopamine Hydrochloride, 160 mg and Dextrose, 5 g per 100 ml; Dopamine Hydrochloride, 320 mg and Dextrose, 5 g per 100 ml.

Identification

A. Dissolve 0.1 g in 10 ml of *water*, add 3 ml of *potassium cupri-tartrate solution*; the solution is blue and clear. Heat to boiling; a copious red precipitate is formed.

B. In the Assay for dopamine hydrochloride, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 2.5 to 4.5.

5-hydroxymethylfurfural and related substances.

Solvent mixture. 0.022 M *sodium hydroxide* in *water*.

Cation-exchange column. A chromatographic tube having a 0.8 cm x 4 cm bed volume (or about 2 ml) of 100 to 200 mesh, strongly acidic, styrene-divinylbenzene cation-exchange resin. Condition the column by washing with about 30 ml of *water*, discarding the eluate.

Pass a volume of injection containing about 100 mg of *hydrous dextrose* through the resin bed in the cation-exchange column, allowing the sample to flow down the wall of the column so as not to disturb the resin bed, and collect the eluate in a 50-ml volumetric flask. Wash the column with 25.0 ml of *water*, and collect the eluate in the same 50-ml volumetric flask. Dilute the eluate with solvent mixture to volume, and mix to obtain the test solution. In a similar manner, prepare a blank by passing 25.0 ml of *water* through a freshly conditioned cation-exchange column, collecting the eluate in a 50-ml volumetric flask, diluting with solvent mixture to volume, and mix. Determine the absorbance of the test solution against the blank in a 1 cm cell at 284 nm (2.4.7). The absorbance obtained is not more than 0.25.

Bacterial endotoxins (2.2.3). Not more than 16.67 Endotoxin Units per mg of dopamine hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay

For dopamine hydrochloride — Determine by liquid chromatography (2.4.14).

Test solution. Mix the content of 10 containers. A measured volume containing 16 mg of Dopamine Hydrochloride to 100-ml volumetric flask, dilute with mobile phase to volume and mix.

Reference solution (a). A 0.016 per cent w/v solution of *dopamine hydrochloride IPRS* in mobile phase.

Reference solution (b). A 2.0 per cent w/v solution of *benzoic acid* in *methanol* and finally dilute with mobile phase to obtain 0.5 per cent w/v solution.

Reference solution (c). Dilute 10.0 ml of the reference solution (a) and 10.0 ml of reference solution (b) to 100.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 4 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 87 volumes of a mixture of 0.005 M *sodium 1-octanesulphonate* in 1 per cent v/v *glacial acetic acid* and 13 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 40 µl.

Inject reference solution (c). The test is not valid unless the resolution between benzoic acid and dopamine hydrochloride is not less than 4.0.

Inject reference solution (a). The relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject reference solution (a) and the test solution.

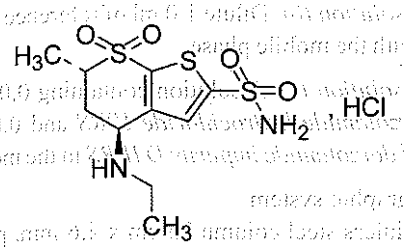
Calculate the content of $C_8H_{11}NO_2,HCl$.

For dextrose — To a measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water*

to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation multiplied by 1.0424 represents the weight, in g, of dextrose, $C_6H_{12}O_6,H_2O$ in the volume taken for assay.

Storage. Store protected from moisture.

Dorzolamide Hydrochloride



$C_{10}H_{16}N_2O_4S_3$, HCl Mol. Wt. 360.9

Dorzolamide Hydrochloride is (4*S*,6*S*)- 4-(ethylamino)-5,6-dihydro-6-methyl-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide, monohydrochloride.

Dorzolamide Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{16}N_2O_4S_3$, HCl, calculated on the anhydrous basis.

Category. Antiglaucoma agent.

Description. A white to off-white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dorzolamide hydrochloride IPRS* or with the reference spectrum of dorzolamide hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Dorzolamide impurity A. Determine by liquid chromatography (2.4.14)

Solvent mixture. 87 volumes of *tert-butyl methyl ether*, 10 volumes of *glacial acetic acid* and 3 volumes of *acetonitrile*.

Test solution. Dissolve 20 mg of the substance under examination in 4 ml of *0.5M ammonium hydroxide* in a centrifuge tube, add 4 ml of *ethyl acetate* and mix. Separate the organic layer and transfer it to a separate centrifuge tube. Add 4 ml of *ethyl acetate* to the aqueous layer, mix, separate the organic layer and combine it with the first extract. Evaporate the combined organic layers to dryness on a water-bath at 50° under a stream of nitrogen. Dissolve the residue in 3 ml of *acetonitrile*, add 3 drops of *(S)-(-)-α-methylbenzyl isocyanate*, and heat on a water-bath at 50° for 5 minutes. Evaporate to dryness on a water-bath at 50° under a stream of nitrogen. Dissolve the residue in 10 ml of the solvent mixture.

Reference solution. Dissolve 18 mg of *dorzolamide hydrochloride IPRS* and 2 mg of *dorzolamide impurity A IPRS ((4R,6R)-4-(ethylamino)-6-methyl-5,6-dihydro-4H-thieno[2,3-b]thiopyran-2-sulphonamide7,7-dioxide, monohydrochloride IPRS)* in 4 ml of *0.5M ammonium hydroxide* in a centrifuge tube and proceed as indicated for the test solution beginning with "add 4 ml of *ethyl acetate*".

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (5µm),
- mobile phase: a mixture of 63 volumes of *tert-butyl methyl ether*, 35 volumes of *n-heptane*, 2 volumes of *acetonitrile* and 0.2 volume of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

The relative retention time with reference to dorzolamide for dorzolamide impurity A is about 1.5.

Inject the reference solution. The test is not valid unless the resolution between the peaks corresponding to dorzolamide and dorzolamide impurity A is not less than 4.0.

Inject the test solution. The area of the peak corresponding to dorzolamide impurity A is not more than 0.5 per cent, calculated by area normalization method.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Inject the test solution. The area of any secondary peak is not more than 0.1 per cent and the sum of areas of all the secondary peaks is not more than 0.5 per cent, calculated by area normalization method.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.4 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 60 mg of the substance under examination in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution. A 0.06 per cent w/v solution of *dorzolamide hydrochloride IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature: 35°,
- mobile phase: A. a mixture of 94 volumes of buffer solution prepared by dissolving 3.7 g of *monobasic potassium phosphate* in 1000 ml of *water* and 6.5 volumes of *acetonitrile*,
  B. *acetonitrile*,
- a gradient program using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
15	100	0
30	50	50
37	100	0
44	100	0

Inject the reference solution. The test is not valid unless the column efficiency is not less than 6500 theoretical plates, the tailing factor is not less than 0.6 and not more than 1.2 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{16}N_2O_4S_3$, HCl.

Storage. Store protected from light and moisture, at a temperature 15° to 30°.

Dorzolamide Eye Drops

Dorzolamide Hydrochloride Eye Drops

Dorzolamide Eye Drops are a sterile solution of Dorzolamide Hydrochloride in Purified Water.

Dorzolamide Eye Drops contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dorzolamide, $C_{10}H_{16}N_2O_4S_3$.

Usual strength. 2 per cent w/v.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 5.0 to 6.0.

Other tests. Comply with the tests stated under Eye Drops.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of eye drops to obtain a solution containing 0.01 per cent w/v of dorzolamide in the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 5.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.011 per cent w/v of *dorzolamide hydrochloride IPRS* and 0.000011 per cent w/v of *dorzolamide impurity D IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1 volume of *acetonitrile* and 19 volumes of a 0.2 per cent v/v solution of *orthophosphoric acid*, adjusted to pH 3.0 with *triethylamine*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 253 nm,
- injection volume: 20 µl.

Name	Relative retention time
Dorzolamide impurity D[1]	0.9
Dorzolamide (retention time: about 11 minutes)	1.0
Dorzolamide impurity B[2]	1.1

[1](4*S*,6*S*)-4-amino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2- sulfonamide 7,7-dioxide,

[2](4*RS*,6*SR*)-4-(ethylamino)-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2- sulfonamide 7,7-dioxide.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to dorzolamide and dorzolamide impurity D is not less than 3.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to dorzolamide impurity B is not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.3 per cent). The area of any peak corresponding to dorzolamide impurity D is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The sum of the areas of all the secondary peaks is not more than 1.5 times

the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the eye drops to obtain a solution containing 0.01 per cent w/v of dorzolamide in the mobile phase.

Reference solution (a). A 0.011 per cent w/v solution of *dorzolamide hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.011 per cent w/v of *dorzolamide hydrochloride IPRS* and 0.000011 per cent w/v of *dorzolamide impurity D IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to dorzolamide and dorzolamide impurity D is not less than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{10}H_{16}N_2O_4S_3$ in the eye drops.

1 mg of $C_{10}H_{16}N_2O_4S_3$,HCl is equivalent to 899 µg of $C_{10}H_{16}N_2O_4S_3$.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of dorzolamide.

Dorzolamide and Timolol Eye Drops

Dorzolamide and Timolol Eye Drops are a sterile solution of Dorzolamide Hydrochloride and Timolol Maleate in Purified Water.

Dorzolamide and Timolol Eye Drops contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount each of dorzolamide, $C_{10}H_{16}N_2O_4S_3$ and timolol, $C_{13}H_{24}N_4O_3S$.

Usual strength. Dorzolamide 2 per cent w/v and timolol 0.5 per cent w/v.

Identification

A. In the Assay for dorzolamide, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

B. In the Assay for timolol, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.0 to 6.0.

Related substances. *For dorzolamide* — Determine by liquid chromatography (2.4.14).

Solvent mixture. 1 volume of *acetonitrile* and 19 volumes of a 0.2 per cent v/v solution of *orthophosphoric acid.*

Test solution. Dilute a volume of eye drops to obtain a solution containing 0.01 per cent w/v of dorzolamide with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 5.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.011 per cent w/v of *dorzolamide hydrochloride IPRS* and 0.000011 per cent w/v each of *dorzolamide impurity B IPRS* and *drozolamide impurity D IPRS.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *acetonitrile,*
  B. 0.2 per cent v/v *orthophosphoric acid,*
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 253 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	5	95
15	5	95
15.1	95	5
20	95	5
20.1	5	95
30	5	95

Name	Relative retention time
Dorzolamide impurity D[1]	0.8
Dorzolamide (retention time: about 12 minutes)	1.0
Dorzolamide impurity B[2]	1.2

[1](4*S*,6*S*)-4-amino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2- sulfonamide 7,7-dioxide,

[2](4*RS*,6*SR*)-4-(ethylamino)-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2- sulfonamide 7,7-dioxide.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to dorzolamide and dorzolamide impurity D is not less than 3.0.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to dorzolamide impurity B is not more than 1.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.1 per cent), the area of any peak due to dorzolamide impurity D is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 1.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and peaks due to timolol and maleic acid.

For timolol — Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of eye drops to obtain a solution containing 0.025 per cent w/v of timolol in the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase. Further dilute 2.0 ml of this solution to 5.0 ml with the mobile phase.

Reference solution (b). Add 8 ml of *0.1 M sodium hydroxide* to 90 mg of *timolol maleate IPRS*, heat at 70° for 15 hours, cool and dilute to 50.0 ml with the mobile phase. Mix 1 volume of the solution with 4 volumes of a 0.14 per cent w/v solution of *dorzolamide hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 2 volumes of *methanol* and 3 volumes of a 1.1 per cent w/v solution of *sodium dihydrogen orthophosphate monohydrate*, adjusted to pH 2.8 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 20 µl.

Name	Relative retention time
Dorzolamide	0.5
Timolol impurity G[1]	0.6
Timolol impurity B[2]	0.7
Timolol (retention time: about 5.7 minutes)	1.0
Timolol impurity D[3]	1.5

[1]4-(morpholin-4-yl)-1,2,5-thiadiazol-3(2*H*)-one 1-oxide,
[2](2*RS*)-3-[(1,1-dimethylethyl)amino]-2-[[4-(morpholin-4-yl)-1,2,5-thiadiazol-3- yl]oxy]propan-1-ol,
[3]4-(morpholin-4-yl)-1,2,5-thiadiazol-3-ol.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to timolol impurity G and timolol impurity B is not less than 1.5.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the timolol peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to timolol impurities B, D or G is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and peaks due to maleic acid.

Other tests. Comply with the tests stated under Eye Drops.

Assay. *For Dorzolamide*—Determine by liquid chromatography (2.4.14).

Solvent mixture. 1 volume of *acetonitrile* and 19 volumes of 0.2 per cent v/v solution of *orthophosphoric acid*.

Test solution. Dilute the eye drops to obtain a solution containing 0.01 per cent w/v of dorzolamide in the solvent mixture.

Reference solution (a). A 0.011 per cent w/v solution of *dorzolamide hydrochloride IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.011 per cent w/v of *dorzolamide hydrochloride IPRS* and 0.000011 per cent w/v of *dorzolamide impurity D IPRS* in the solvent mixture.

Use chromatographic system as described under Related substances for dorzolamide.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to dorzolamide and dorzolamide impurity D is not less than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{10}H_{16}N_2O_4S_3$ in the eye drops.

1 mg of $C_{10}H_{16}N_2O_4S_3$,HCl is equivalent to 899 µg of $C_{10}H_{16}N_2O_4S_3$.

For timolol — Determine by liquid chromatography (2.4.14).

Test solution. Dilute the eye drops to obtain a solution containing 0.025 per cent w/v of timolol in the mobile phase.

Reference solution (a). A 0.0342 per cent w/v solution of *timolol maleate IPRS* in the mobile phase.

Reference solution (b). Add 8 ml of *0.1 M sodium hydroxide* to 90 mg of *timolol maleate IPRS*, heat at 70° for 15 hours, cool and dilute to 50.0 ml with the mobile phase. Mix 1 volume of the solution with 4 volumes of a 0.14 per cent w/v solution of *dorzolamide hydrochloride IPRS*.

Use chromatographic system as described under Related substances for timolol.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to timolol impurity G and timolol impurity B is not less than 1.5.

Inject reference solution (a) and the test solution.

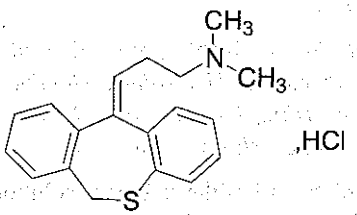
Calculate the content of $C_{13}H_{24}N_4O_3S_3$, in the eye drops.

1 mg of $C_{13}H_{24}N_4O_3S_3,C_4H_4O_4$ is equivalent to 731.6 µg of $C_{13}H_{24}N_4O_3S_3$.

Labelling. The label states the quantity of Dorzolamide Hydrochloride in terms of the equivalent amount of dorzolamide and the quantity of Timolol Maleate in terms of the equivalent amount of timolol.

Dothiepin Hydrochloride

Dosulepin Hydrochloride



$C_{19}H_{21}NS,HCl$ Mol. Wt. 331.9

Dothiepin Hydrochloride is 3-(6*H*-dibenzo[*b,e*]thiepin- 11-ylidene) propyldimethylamine hydrochloride, consisting predominantly of the E-isomer.

Dothiepin Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{21}NS,HCl$, calculated on the dried basis.

Category. Antidepressant

Description. A white to faintly yellow, crystalline powder.

Identification

Tests B and C may be omitted if tests A and D are carried out. Test A may be omitted if tests B, C and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dothiepinhydrochloride IPRS* or with the reference spectrum of dothiepin hydrochloride.

B. When examined in the range 220 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in 0.1 per cent v/v solution of *methanolic hydrochloric acid* shows absorption maxima at about 231 nm and 306 nm; specific absorbance at 231 nm is 660 to 730.

C. Dissolve 1 mg in 5 ml of *sulphuric acid;* a dark red colour is produced.

D. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1) and not more intensely coloured than reference solution YS5 (2.4.1).

pH (2.4.24). 4.2 to 5.2, determined in a 10.0 per cent w/v solution in *carbon dioxide-free water.*

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light and use freshly prepared solutions.

Test solution. Dissolve 50 mg of the substance under examination in 5 ml of *methanol* and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 12.5 mg of *dothiepin impurity A IPRS* in 5 ml of *methanol* and dilute to 50.0 ml with the mobile phase. Dilute 0.5 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *dothiepin for system suitability IPRS* (containing impurity E) in 5 ml of *methanol* and dilute to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with cyanosilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture 1 volume of 0.83 per cent v/v solution of *perchloric acid*, 10 volumes of *propanol*, 30 volumes of *methanol* and 60 volumes of *water,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 229 nm,
- injection volume: 5 µl.

Name	Relative retention time
Dothiepin impurity A[1]	0.3
Dothiepin (Retention time: about 14 minutes)	1.0
Dothiepin impurity E[2]	0.92

[1](*E*)-3-(5-oxo-5λ4-dibenzo[*b,e*]thiepin-11(6*H*)-ylidene)-*N,N*-dimethylpropan-1-amine,

[2](*Z*)-3-(dibenzo[*b*,*e*]thiepin-11(6*H*)-ylidene)-*N*,*N*-dimethylpropan-1-amine.

Inject reference solution (b). Adjust the sensitivity of the system so that the peak-to-valley ratio is minimum 4, where H_p is height above the baseline of the peak due to impurity E and H_V is the height above the baseline of the lowest point of the curve separating this peak from the peak due to dothiepin.

Inject reference solution (a) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak, the area of any secondary peak corresponding to dothiepin impurity E is not more than 5 per cent of the sum of the areas of the peak due to dothiepin impurity E and the principal peak in the chromatogram obtained with the test solution (5 per cent), the area of any peak corresponding to dothiepin impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent), the area of any other secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks other than dothiepin impurity E is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.41 9). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.250 g in a mixture of 5 ml of *anhydrous acetic acid* and 35 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1M perchloric acid* is equivalent to 0.03319 g of $C_{19}H_{21}NS,HCl$

Storage. Store protected from light.

Dothiepin Capsules

Dothiepin Hydrochloride Capsules; Dosulepin Capsules; Dosulepin Hydrochloride Capsules.

Dothiepin Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dothiepin hydrochloride, $C_{19}H_{21}NS,HCl$.

Usual strength. 25 mg.

Identification

Extract a quantity of the contents of the capsules containing 0.1 g of Dothiepin Hydrochloride with 20 ml of *ethanol*, filter and remove the ethanol from the filtrate by evaporation. The residue complies with the following tests.

A. Dissolve 1 mg in 5 ml of *sulphuric acid*; a dark red colour is produced.

B. On 20 mg determine by the oxygen-flask method (2.3.34), using a mixture of 15 ml of *water* and 1 ml of *hydrogen peroxide solution (20 volume)* as the absorbing liquid. The solution gives the reactions of sulphates (2.3.1).

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 90 volumes of *1,2-dichloroethane*, 10 volumes of *2-propanol* and 1 volume of *strong ammonia solution*.

Test solution (a). Extract a quantity of the contents of the capsules containing about 0.25 g of Dothiepin Hydrochloride by shaking for 2 minutes with 5 ml of *chloroform*, centrifuge and use the supernatant liquid.

Test solution (b). Dilute 2.0 ml of test solution (a) to 5.0 ml with *chloroform*.

Reference solution. A freshly prepared solution containing 0.02 per cent w/v each of *11-(3-dimethylamino-propylidene)-6H-dibenzo[b,e]thiepin-5-oxide IPRS* and *6H-dibenzo-[b,e]thiepin-11-one IPRS* in *chloroform*.

Apply to the plate 5 µl of the reference solution and 10 µl of the test solutions. After development, dry the plate in air and examine under ultraviolet light at 254 nm. In the chromatogram obtained with the reference solution the spot with the lower R_f value is more intense than any corresponding spot in the chromatogram obtained with test solution (b). In the chromatogram obtained with test solution (a) any secondary spot other than any spot corresponding to the spot with the lower R_f value in the chromatogram obtained with the reference solution is not more intense than the proximate spot in the chromatogram obtained with the reference solution.

Z-Isomer. Determine by gas chromatography (2.4.13).

Test solution. Use the supernatant liquid obtained by extracting a quantity of the mixed contents of 20 capsules containing 25 mg of Dothiepin Hydrochloride with 5.0 ml of *methanol* and centrifuging.

Reference solution. A 0.5 per cent w/v solution of *dothiepin hydrochloride IPRS* in *methanol*.

Chromatographic system
- a glass column 1.8 m x 3 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (Such as OV-225),
- temperature:
  column.200°,
  inlet port. 260°,
- flame ionisation detector,
- nitrogen as the carrier gas.

In the chromatogram obtained with the reference solution a peak due to Z-dothiepin is present with a retention time of approximately 0.83 relative to the retention time of the principal peak which is due to *E*-dothiepin. In the chromatogram obtained with the test solution the area of any peak corresponding to Z-dothiepin is not greater than 7.5 per cent of the sum of the areas of the peaks due to Z-dothiepin and *E*-dothiepin.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 0.5 g of Dothiepin Hydrochloride and extract with 20 ml followed by four quantities, each of 10 ml, of *chloroform*, filtering each extract through the same filter. Evaporate the combined extracts to dryness, dissolve the residue in 100 ml of *acetone* and add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03319 g of $C_{19}H_{21}NS,HCl$.

Storage. Store protected from moisture.

Dothiepin Tablets

Dothiepin Hydrochloride Tablets; Dosulepin Tablets; Dosulepin Hydrochloride Tablets

Dothiepin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dothiepin hydrochloride, $C_{19}H_{21}NS,HCl$.

Usual strengths. 25 mg; 50 mg; 75 mg; 100 mg; 150 mg.

Identification

Extract a quantity of the powdered tablets containing about 0.2 g of Dothiepin Hydrochloride with 20 ml of *dichloromethane*, centrifuge, filter through *anhydrous sodium sulphate* and evaporate the filtrate to dryness. Dissolve the residue in the minimum quantity of *ethanol* and add an excess of *ether*. Filter the precipitate, wash with *ether* and dry. The residue complies with the following tests.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dothiepin hydrochloride IPRS* or with the reference spectrum of dothiepin hydrochloride.

Tests

Related substances. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *13.5 M ammonia*, 10 volumes of *2-propanol* and 90 volumes of *1,2-dichloroethane*

Test solution. Disperse a quantity of the powdered tablets containing 0.25 g of Dothiepin Hydrochloride with 5 ml of *dichloromethane*, centrifuge and use the clear supernatant liquid.

Reference solution (a). Dilute 2.0 ml of the test solution to 5.0 ml with *dichloromethane*.

Reference solution (b). A solution containing 0.010 per cent w/v each of *3-(dibenzo[b,e]thiepin-11(6H)-ylidene)-N,N-dimethylaminopropan-1-amine S-oxide hydrochloride IPRS* and *3-(dinbenzo[b,e]thiepin-11(6H)-one IPRS* in *chloroform*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise about three-fourths of the height of the plate. Dry the plate in air and examine under ultraviolet light at 254 nm. In the chromatogram obtained with reference solution (b) the spot with the lower R_f value is more intense than any corresponding spot in the chromatogram obtained with reference solution (a) (0.5 per cent). In the chromatogram obtained with test solution, any secondary spot other than any spot corresponding to the spot with the lower R_f value in the chromatogram obtained with reference solution (b) is not more intense than the proximate spot in the chromatogram obtained with reference solution (b) (0.2 per cent)

Z-Impurity. Determine by gas chromatography (2.4.13).

Test solution. Disperse a quantity of tablet powder containing 25 mg of the Dothiepin Hydrochloride in 5.0 ml of *methanol* for 15 minutes, centrifuge and use the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of *dothiepin hydrochloride IPRS* in *methanol*.

Chromatographic system
- a glass column 1.8 m x 3 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (Such as OV-225),
- temperature:
  column. 200°,
  inlet port. 260°,
- flame ionisation detector,
- nitrogen as the carrier gas,
- injection volume: 1 µl.

In the chromatogram obtained with the reference solution a peak due to Z-dothiepin is present with a retention time of approximately 0.83 relative to the retention time of the principal peak which is due to *E*-dothiepin. In the chromatogram obtained with the test solution the area of any peak corresponding to Z-dothiepin is not more than 7.5 per cent of the sum of the areas of the peaks due to Z-dothiepin and *E*-dothiepin.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Dothiepin Hydrochloride with about 70 ml of *0.1 M hydrochloric acid*, shake for about 30 minutes and dilute to 100.0 ml with *0.1 M hydrochloric acid* and filter, rejecting the first few ml of filtrate. Dilute 25.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*.

Reference solution. A 0.0125 per cent w/v solution of *dothiepin hydrochloride IPRS* in *0.1 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *tetrahydrofuran*, 40 volumes of *acetonitrile*, and 50 volumes of 0.5 per cent w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *2M orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 231 nm,
- injection volume: 20 µl.

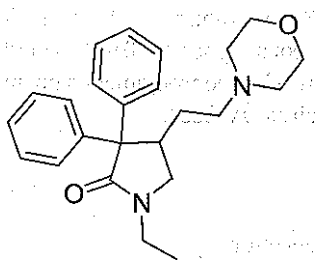
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content, $C_{19}H_{21}NS,HCl$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Doxapram Hydrochloride



, HCl , H_2O

$C_{24}H_{30}N_2O_2,HCl,H_2O$ Mol. Wt. 433.00

Doxapram Hydrochloride is (*RS*)-1-Ethyl-4-(2-morpholinoethyl)-3,3-diphenyl-2-pyrrolidone hydrochloride monohydrate.

Doxapram Hydrochloride contains not less than 98.0 per cent and not more than 100.5 per cent of $C_{24}H_{30}N_2O_2,HCl$, calculated on the dried basis.

Category. Respiratory stimulant.

Description. A white or almost white, crystalline powder.

Identification

Test B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *doxapram hydrochloride IPRS* or with the reference spectrum of doxapram hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 10 volumes of 1.7 per cent w/v of *ammonia*, 10 volumes of *2-propanol* and 80 volumes of *2- methylpropanol*.

Test solution. Dissolve 10 mg of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol*.

Reference solution. A 0.1 per cent w/v solution of *doxapram hydrochloride IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with *dilute potassium iodobismuthate solution* and examine immediately. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 3.5 to 5.0, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

Optical rotation (2.4.22). - 0.1° to + 0.1°, determined on 5.0 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 5.0 ml with the mobile phase.

Reference solution (c). A 0.1 per cent w/v solution of *doxapram impurity B IPRS ((4RS)-1-ethyl-4-[2-[(2-hydroxyethyl) amino]ethyl]-3,3-diphenylpyrrolidin-2-one IPRS)* in the mobile phase. To 1.0 ml of the solution, add 1.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.082 per cent w/v solution of *sodium acetate,* adjusted to pH 4.5 with *glacial acetic acid,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to doxapram and doxapram impurity B is not less than 3.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). Dissolve 2.0 g in a mixture of 15 volumes of *water* and 85 volumes of *methanol* and dilute to 20 ml with the same solvent mixture. 12 ml of the solution complies with limit test for heavy metals, Method D (20 ppm), using 10.0 ml of *lead standard solution (2 ppm Pb).*

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 3.0 per cent to 4.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.3 g in a mixture of 10 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (95 per cent).* Titrate with *0.1 M sodium hydroxide,* determining the end point potentiometrically (2.4.25). Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0415 g of $C_{24}H_{31}ClN_2O_2$.

Doxapram Injection

Doxapram Hydrochloride Injection

Doxapram Injection is a sterile solution of Doxapram Hydrochloride in Water for Injections.

Doxapram Injection contains not less than 90.0 per cent and not more than 110.0 per cent of doxapram hydrochloride, $C_{24}H_{30}N_2O_2,HCl,H_2O$.

Usual strength. 20 mg per ml.

Identification

A. To a volume containing 50 mg of Doxapram Hydrochloride, add 10 ml of *water* and 2 ml of *1 M sodium hydroxide* and extract with two 10 ml quantities of *ether.* Wash the combined extracts with 5 ml of *water,* dry over *anhydrous sodium sulphate,* filter and evaporate to dryness. Recrystallise the residue from 10 ml of *0.01M methanolic hydrochloric acid.* On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *doxapram hydrochloride IPRS,* treated in similar manner or with the reference spectrum of doxapram.

B. When examined in the range 230 nm to 350 nm (2.4.7) of the solution obtained in the Assay exhibits maxima at about 253 nm, 258 nm and 265 nm.

C. It gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 5.0, determined in a 1.0 per cent w/v solution of doxapram hydrochloride.

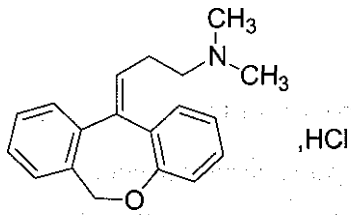
Bacterial endotoxins (2.2.3). Not more than 3.3 Endotoxin Units per mg of doxapram hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (injections).

Assay. Dilute a volume containing 0.2 g of Doxapram Hydrochloride to 250 ml with *water.* Measure the absorbance of the resulting solution at the maximum at 258 nm (2.4.7). Calculate the content of $C_{24}H_{30}N_2O_2,HCl,H_2O$ in the injection from the absorbance of a 0.08 per cent w/v solution of *doxapram hydrochloride IPRS.*

Storage. It should not be allowed to freeze.

Doxepin Hydrochloride



$C_{19}H_{21}NO,HCl$ Mol. Wt. 315.8

Doxepin Hydrochloride is 3-(6*H*-dibenz[*b*,*e*]oxepin-11- ylidene) propyldimethylamine hydrochloride. It consists of a mixture of *Z* and *E* isomers.

Doxepin Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{21}NO,HCl$, calculated on the dried basis.

Category. Antidepressant.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *doxepin hydrochloride IPRS* or with the reference spectrum of doxepin hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.01 M methanolic hydrochloric acid* shows an absorption maximum only at 297 nm, about 0.60.

C. Dissolve 5 mg in 2 ml of *nitric acid*; a red colour is produced.

D. It gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use and protect them from light.

Solvent mixture. 1 volume of *1 M sodium hydroxide* and 250 volumes of the mobile phase.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *acetonitrile* and 30 volumes of phosphate buffer solution prepared by dissolving 1.42 g of *anhydrous disodium hydrogen phosphate* in *water*, adjusted to pH 7.7 with *orthophosphoric acid* and dilute to 1000 ml with *water* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Doxepin impurity A[1]	0.5	—
Doxepin impurity C[2]	0.6	—
Doxepin impurity B[3]	0.7	1.7
Doxepin (Retention time: about 18 minutes)	1.0	—

[1]doxepinone,

[2]desmethyldoxepin,

[3]doxepinol.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to doxepin impurities A, B is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any peak corresponding to doxepin impurity C is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

***Z*-Isomer.** Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12 cm x 4 mm, packed with octylsilane bonded to porous silica (5 µm) with a specific surface area of 220 m^2/g and a pore size of 80 nm,
- column temperature: 50°,
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of 3.0 per cent w/v solution of *sodium dihydrogen phosphate* previously adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the test solution. The test is not valid unless the resolution between the peaks due to *E*-isomer (1st peak) and to the *Z*-isomer (2nd peak) is not less than 1.5. Calculate the ratio of the area of the peak due to the *E*-isomer to the area of the peak due to the *Z*-isomer: this ratio is 4.4 to 6.7 (13.0 per cent to 18.5 per cent of the *Z*-isomer).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.6 g in 100 ml of *acetone* and add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03158 g of $C_{19}H_{21}NO,HCl$.

Storage. Store protected from light.

Doxepin Capsules

Doxepin Hydrochloride Capsules

Doxepin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of doxepin, $C_{19}H_{21}NO$.

Usual strengths. 25 mg; 50 mg and 75 mg.

Identification

Wash a quantity of the contents of the capsules containing 0.1 g of doxepin with 3 quantities, each of 5 ml, of *light petroleum (40° to 60°)*. Dry the residue in air and extract with 3 quantities, each of 10 ml, of *chloroform*, evaporate the combined extracts to dryness and dry the residue at 105°. The dried residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *doxepin hydrochloride IPRS* or with the reference spectrum of doxepin hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.01 M methanolic hydrochloric acid* shows an absorption maximum only at about 297 nm; absorbance at about 297 nm, about 0.60.

C. Dissolve 5 mg in 2 ml of *nitric acid*; a red colour is produced.

D. It gives reaction (A) of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 1(Basket),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 292 nm (2.4.7). Calculate the content of $C_{19}H_{21}NO$ in the medium from the absorbance obtained from a solution of known concentration of *doxepin hydrochloride IPRS* in the same medium.

Q. Not less than 80 per cent of the stated amount of $C_{19}H_{21}NO$.

***Z*-Isomer**. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the mixed contents of 20 capsules containing 20 mg of doxepin in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12 cm x 4 mm, packed with octylsilane bonded to porous silica (5 µm) with a specific surface area of 220 m^2/g and a pore size of 80 nm,
- column temperature: 50°,
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of 3.0 per cent w/v solution of *sodium dihydrogen phosphate* previously adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the test solution. The test is not valid unless the resolution between the peaks due to *E*-isomer (first peak) and to the *Z*-isomer (second peak) is not less than 1.5. Calculate the ratio of the area of the peak due to the *E*-isomer to the area of the peak due to the *Z*-isomer: this ratio is 4.4 to 6.7 (13.0 per cent to 18.5 per cent of the *Z*-isomer).

Other tests. Comply with the tests stated under Capsules.

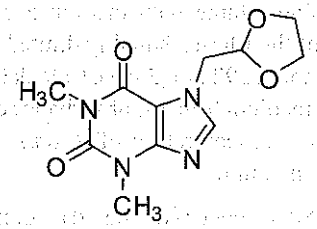
Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 30 mg of doxepin, add 50 ml of *0.1 M methanolic hydrochloric acid*, shake for 30 minutes and add

sufficient *0.01 M methanolic hydrochloric acid* to produce 100.0 ml. Centrifuge 40 ml of the solution and dilute 10.0 ml of the clear supernatant liquid to 100.0 ml with *0.01 M methanolic hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 297 nm (2.4.7). Calculate the content of $C_{19}H_{21}NO$ taking 150 as the specific absorbance at 297 nm.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of doxepin.

Doxofylline



$C_{11}H_{14}N_4O_4$ Mol. Wt. 266.3

Doxofylline is 7-(1,3-dioxolan-2-ylmethyl)-3,7-dihydro-1,3-dimethyl-1*H*-purine-2,6-dione.

Doxofylline contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{11}H_{14}N_4O_4$, calculated on the dried basis.

Category. Bronchodilator.

Description. A white to off white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *doxofylline IPRS* or with the reference spectrum of doxofylline.

B. When examined in the range of 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *water* shows an absorption maximum at about 274 nm.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A solution containing 0.05 per cent w/v each of *doxofylline IPRS* and *theophylline IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Cosmosil C18),
- mobile phase: a mixture of 80 volumes of *water* and 20 volumes of *acetonitrile*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to theophylline and doxofylline is not less than 5.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to theophylline is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the area of any other secondary peak is not more than the area of the peak in the chromatogram obtained reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained reference solution (b) (1.0 per cent).

Heavy Metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 40 mg of substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.004 per cent w/v solution of *doxofylline IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Cosmosil C18),
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, and 20 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{14}N_4O_4$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Doxofylline Tablets

Doxofylline Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of doxofylline, $C_{11}H_{14}N_4O_4$.

Usual strength. 400 mg.

Identification

A. Extract a quantity of the powdered tablets containing about 0.1 g of Doxofylline with 40 ml of *chloroform*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained from *doxofylline IPRS* or with the reference spectrum of doxofylline.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtered solution, suitably diluted if necessary with the medium, at the maximum at about 275 nm (2.4.7). Calculate the content of $C_{11}H_{14}N_4O_4$ in the medium from the absorbance obtained from a solution of known concentration of *doxofylline IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{11}H_{14}N_4O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing about 25 mg of Doxofylline in 25.0 ml in the mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of *doxofylline IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Use chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Weigh and powder 20 tablets. Disperse a quantity of powder containing about 50 mg of Doxofylline in 70 ml of *methanol*, sonicate for 15 minutes and dilute to 100.0 ml with *methanol*, filter.

Test solution (b). Dilute 2.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *doxofylline IPRS* in *methanol*.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *water* and 70 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume: 20 µl.

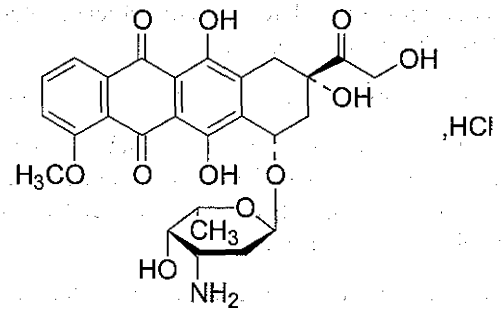
Inject reference solution (b). The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and test solution (b).

Calculate the content of $C_{11}H_{14}N_4O_4$ in the tablets.

Storage. Store protected from light and moisture.

Doxorubicin Hydrochloride



$C_{27}H_{29}NO_{11},HCl$ Mol. Wt. 580.0

Doxorubicin Hydrochloride is (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-*L*-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-hydroxyacetyl-1-methoxy-7,8,9,10-tetrahydronaphthacene-5,12-dione hydrochloride, a substance produced by the growth of certain strains of *Streptomyces coeruleorubidus* or *S. peucetius* or obtained by any other means.

Doxorubicin Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{27}H_{29}NO_{11},HCl$, calculated on the anhydrous and solvent-free basis.

Category. Cytotoxic.

Description. An orange-red, crystalline powder; hygroscopic.

CAUTION — Doxorubicin Hydrochloride is poisonous. It must be handled with care avoiding contact with skin and inhalation of airborne particles.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6) Compare the spectrum with that obtained with *doxorubicin hydrochloride IPRS* or with the reference spectrum of doxorubicin hydrochloride.

B. In the test for Related substances, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (c).

C. Dissolve 10 mg in 0.5 ml of *nitric acid,* add 0.5 ml of *water* and heat over a flame for 2 minutes. Allow to cool and add 0.5 ml of *silver nitrate solution;* a white precipitate is produced.

Tests

pH (2.4.24). 4.0 to 5.5, determined in a 0.5 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in sufficient of the mobile phase to produce 50 ml.

Test solution (b). Dilute 10.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 10 mg of *doxorubicin hydrochloride IPRS* and 10 mg of *epirubicin hydrochloride IPRS* in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 5 ml of reference solution (a) to 20 ml with the mobile phase.

Reference solution (c). Dissolve 50 mg of *doxorubicin hydrochloride IPRS* in the mobile phase and dilute to 50 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *acetonitrile* and a solution containing 2.88 g per litre of *sodium dodecylsulphate* and 2.25 g per litre of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (a), (b) and test solution (a). Continue the chromatography for 3.5 times the retention time of doxorubicin of about 8 minutes. The test is not valid unless in the chromatogram obtained with reference solution (a) the resolution factor between the peaks due to doxorubicin and epirubicin is at least 2.0.

In the chromatogram obtained with the test solution the area of any peak other than the principal peak is not more than the area of the peak corresponding to doxorubicin in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the peak corresponding to doxorubicin in the chromatogram obtained with reference solution (b) (0.05 per cent).

Acetone and ethanol. Not more than 2.0 per cent w/w together of which not more than 0.5 per cent w/w is acetone, determined by gas chromatography (2.4.13), injecting 1 µl of each of two solutions

Test solution. A 5.0 per cent w/v solution of the substance under examination and 0.1 per cent w/v of *dioxon* in *water*.

Reference solution. 0.05 per cent w/v of *acetone*, 0.05 per cent w/v of *ethanol* and 0.1 per cent w/v of the internal standard.

Chromatographic system

- a glass column 2 m x 3 mm, packed with acid-washed diatomaceous support (180 to 250 mesh) impregnated with 10 per cent w/w of *polyethylene glycol 20,000* (Such as Carbowax 20M or Chromosorb E/AW),

- temperature:
  column.70°,
  inlet port and detector. 125°,
- flow rate: 30 ml per minute, using helium or nitrogen as the carrier gas.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14) by the procedure described under the test for Related substances.

Inject reference solution (c) and test solution (b).

Calculate the content of $C_{27}H_{29}NO_{11}$,HCl.

Doxorubicin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 2.2 Endotoxin Units per mg.

Doxorubicin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture. If the material is sterile, it should be stored in sterile, tamper-evident containers and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Doxorubicin Injection

Doxorubicin Hydrochloride Injection

Doxorubicin Injection is a sterile solution of Doxorubicin Hydrochloride in Water for Injections made isotonic with Sodium Chloride, Dextrose or other suitable added substances. It is either supplied as preformed solution or it is prepared by dissolving the contents of a sealed container containing Doxorubicin Hydrochloride with or without auxiliary substances in the requisite amount of Water for Injections or Sodium Chloride Injection as directed on the label.

Doxorubicin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of doxorubicin hydrochloride, $C_{27}H_{29}NO_{11}$,HCl.

Usual strength. 2 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 2.5 to 4.5 for the preformed solution and 4.5 to 6.5, determined in the injection prepared in accordance with the directions on the label.

Bacterial endotoxins (2.2.3). Not more than 2.2 Endotoxin Units per mg of doxorubicin hydrochloride, determined in a solution prepared by diluting the injection, if necessary, with *water BET* to obtain a concentration of 2.0 mg of doxorubicin hydrochloride per ml.

Sterility. Complies with the test for sterility, Method A, (2.2.11), using the entire contents of all the containers collected aseptically.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A solution containing 0.05 per cent w/v of doxorubicin hydrochloride prepared by diluting a measured volume of the injection containing not less than 2 mg of Doxorubicin Hydrochloride with the mobile phase or by dissolving the contents of the sealed container in sufficient mobile phase to give a solution of the same strength.

Reference solution (a). A 0.05 per cent w/v solution of *doxorubicin hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v each of *doxorubicin hydrochloride IPRS* and *epirubicin hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a solution containing 0.288 per cent w/v of *sodium dodecyl sulphate* and 0.23 per cent w/v of *phosphoric acid*, 45 volumes of *acetonitrile* and 5 volumes of *methanol*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to doxorubicin and epirubicin is not less than 2.0 in the chromatogram obtain with reference solution (b) and the relative standard deviation for replicate injections is not more than 1.0 per cent in the chromatogram obtain with reference solution (a).

Inject reference solution (a) and test solution.

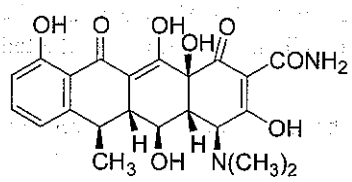
Calculate the content of $C_{27}H_{29}NO_{11}$,HCl in the injection.

Storage. Store the sealed container at a temperature not exceeding 30°. Store the preformed solution protected from light in a refrigerator. Use the solution prepared in the liquid

stated on the label immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the instructions of the manufacturer.

Doxycycline Hydrochloride

Doxycycline Hyclate



,HCl, $^1/_2$ C_2H_5OH, $^1/_2$ H_2O

$C_{22}H_{24}N_2O_8$,HCl,½C_2H_6O,½H_2O Mol. Wt. 513.0

Doxycycline Hydrochloride is (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12, 12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide hydrochloride hemiethanolate hemihydrate, an antimicrobial substance obtained from oxytetracycline or methacycline or by any other means.

Doxocycline Hydrochloride is not less than 95.0 per cent and not more than 102.0 per cent of $C_{22}H_{25}ClN_2O_8$, calculated on anhydrous and ethanol free basis.

Category. Antibacterial.

Description. A yellow, crystalline powder; hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *doxycycline hydrochloride IPRS* or with the reference spectrum of doxycycline hydrochloride.

B. Determine by thin-layer chromatography (2.4.7), coating the plate with *silica gel H*.

Mobile phase. A mixture of 59 volumes of *dichloromethane*, 35 volumes of *methanol* and 6 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *doxycycline hydrochloride IPRS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v each of *doxycycline hydrochloride IPRS* and *tetracycline hydrochloride IPRS* in *methanol*.

Spray the plate evenly with a 10 per cent w/v solution of *disodium edetate* the pH of which has been adjusted to 9.0 with *10 M sodium hydroxide*. Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour. Apply to the plate 1 µl of each solution. After development, dry the plate in a current of air and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. To about 2 mg add 5 ml of *sulphuric acid*; a yellow colour is produced.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 2.0 to 3.0, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). –120° to –105°, calculated on anhydrous and ethanol free basis. Determined within 5 minutes of preparing, in a 1.0 per cent w/v solution in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol*.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol*, measured within 1 hour of preparing the solution, at the maximum at about 349 nm, 0.300 to 0.335, calculated on anhydrous and ethanol free basis.

Light-absorbing impurities. Dissolve 0.1 g in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce 10 ml. Absorbance of the solution at about 490 nm (2.4.7), when measured within 1 hour of preparing the solution, not more than 0.07, calculated on anhydrous and ethanol free basis.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 80 mg of the substance under examination in 100 ml of *0.01 M hydrochloric acid*.

Reference solution (a). A 0.08 per cent w/v solution of *doxycycline hydrochloride IPRS* in *0.01 M hydrochloric acid*.

Reference solution (b). A 0.08 per cent w/v solution of *6-epidoxycycline hydrochloride IPRS* in *0.01 M hydrochloric acid*.

Reference solution (c). A 0.08 per cent w/v solution of *methacycline hydrochloride IPRS* in *0.01 M hydrochloric acid*.

Reference solution (d). A solution containing 0.0016 per cent w/v each of *6-epidoxyccline hydrochloride IPRS* and

methacycline hydrochloride IPRS in *0.01 M hydrochloric acid.*

Reference solution (e). Dilute a mixture of 4 volumes of reference solution (a), 1.5 volumes of reference solution (b) and 1 volume of reference solution (c) to 25 volumes with *0.01 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to10 µm),
- column temperature. 60°,
- mobile phase: a solution prepared by adding 60 g of *2-methyl-2-propanol* to a volumetric flask with the aid of 200 ml of *water*, adding 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1 per cent w/v solution of *tetrabutyl ammonium hydrogen sulphate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and 10 ml of a 4 per cent w/v solution of *disodium edetate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Using reference solution (e). The test is not valid unless (a) the resolution between the first peak (methacycline) and the second peak (6-epidoxycycline) is at least 1.25, (b) the resolution between the second peak and the third peak (doxycycline) is at least 2.0 (adjust the content of 2-methyl-2-propanol in the mobile phase if necessary) and (c) the tailing factor for the third peak is at most 1.25.

Inject reference solution (a). The test is not valid unless the relative standard deviation of the area of the peak due to doxycycline is not more than1.0 per cent.

Inject the test solution and reference solution (d). In the chromatogram obtained with the test solution the area of any peak corresponding to methacycline or 6-epidoxycycline is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (d); the area of any peak appearing between the solvent peak and the peak corresponding to methacycline and the area of any peak appearing on the tail of the main peak is not greater than 25 per cent of that of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with reference solution (d).

Ethanol. 4.3 to 6.0 per cent w/w of C_2H_6O.

Determine by gas chromatography (2.4.13).

Test solution (a). A 1 per cent w/v solution of the substance under examination in a 0.05 per cent v/v solution of *1-propanol* (internal standard) in *water* (solution A).

Test solution (b). A 1 per cent w/v solution of the substance under examination in *water*.

Reference solution. A 0.05 per cent v/v solution of *ethanol* in solution A.

Chromatographic system
- a column 1.5m x 4 mm, packed with porous polymer beads (80 to 100 mesh) (Such as Porapak Q),
- temperature:
  column.135°,
  inlet port and detector. 150°,

Calculate the content of C_2H_6O taking 0.790 g as its weight per ml (2.4.29) at 20°.

Heavy metals (2.3.13). 0.4 g complies with the limit test for heavy metals, Method B (50 ppm).

Sulphated ash (2.3.18). Not more than 0.4 per cent.

Water (2.3.43). 1.4 to 2.8 per cent, determined on 1.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 80 mg of the substance under examination and dissolve in 100 ml of *0.01 M hydrochloric acid.*

Reference solution. A 0.08 per cent w/v solution of *doxycycline hydrochloride IPRS* in *0.01 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
- column temperature: 60°
- mobile phase: a solution prepared by adding 60 g of *2-methyl-2-propanol* to a volumetric flask with the aid of 200 ml of *water*, adding 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1 per cent w/v solution of *tetrabutyl ammonium hydrogen sulphate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and 10 ml of a 4 per cent w/v solution of *disodium edetate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{25}ClN_2O_8$.

Doxycycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.14 Endotoxin Units per mg.

Doxycycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture at a temperature not exceeding 30°. If the substance is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states, where applicable, that the material is sterile.

Doxycycline Capsules

Doxycycline Hydrochloride Capsules

Doxycycline Capsules contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of doxycycline, $C_{22}H_{24}N_2O_8$.

Usual strengths. The equivalent of 50 mg; 100 mg; 200 mg of doxycycline.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

Mobile phase. A mixture of 59 volumes of *dichloromethane*, 35 volumes of *methanol* and 6 volumes of *water.*

Test solution. Shake a quantity of the contents of the capsules containing 50 mg of *anhydrous doxycycline* with 100 ml of *methanol* for 1 to 2 minutes, centrifuge and use the supernatant liquid. Prepare freshly.

Reference solution (a). A 0.05 per cent w/v solution of *doxycycline hydrochloride IPRS* in *methanol.*

Reference solution (b). A solution containing 0.05 per cent w/v each of *doxycycline hydrochloride IPRS* and *tetracycline hydrochloride IPRS* in *methanol.*

Spray the plate evenly with a 10 per cent w/v solution of *disodium edetate* the pH of which has been adjusted to 9.0 with *10 M sodium hydroxide.* Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour. Apply to the plate 1 µl of each solution. After development, dry the plate in a current of air and examine it under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

B. To 0.5 mg of the contents of the capsules add 2 ml of *sulphuric acid*; a yellow colour is produced.

C. A 5 per cent w/v solution of the contents of the capsules gives the reactions of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate suitably diluted with the medium, if necessary, at the maximum at about 345 nm. (2.4.7). Calculate the content of $C_{22}H_{24}N_2O_8$ in the medium from the absorbance obtained from a solution of known concentration of *doxycycline hydrochloride IPRS* in the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{22}H_{24}N_2O_8$.

Light-absorbing impurities. Dissolve the contents of 5 capsules as completely as possible in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce a solution containing the equivalent of 1.0 per cent w/v of anhydrous doxycycline and filter. Absorbance of the filtrate at about 490 nm, not greater than 0.2 (2.4.7), calculated with reference to the dried contents of the capsules.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the contents of the capsules containing 7 mg of anhydrous doxycycline in 10 ml of *0.01 M hydrochloric acid*, filter and use the filtrate.

Reference solution (a). A 0.08 per cent w/v solution of *doxycycline hydrochloride IPRS* in *0.01 M hydrochloric acid.*

Reference solution (b). A 0.08 per cent w/v solution of *6-epidoxycycline hydrochloride IPRS* in *0.01 M hydrochloric acid.*

Reference solution (c). A 0.08 per cent w/v solution of *methacycline hydrochloride IPRS* in *0.01 M hydrochloric acid.*

Reference solution (d). A solution containing 0.0016 per cent w/v each of *6-epidoxycycline hydrochiloride IPRS* and *methacycline hydrochloride IPRS* in *0.01 M hydrochloric acid.*

Reference solution (e). Dilute a mixture of 4 volumes of reference solution (a), 1.5 volumes of reference solution (a) and 1 volume of reference solution (c) to 25 volumes with *0.01 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
- column temperature: 60°,
- mobile phase: a solution prepared by adding 60 g of *2-methyl-2-propanol* to a volumetric flask with the aid of 200 ml of *water*, adding 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1 per cent w/v solution of *tetrabutyl ammonium hydrogen sulphate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and 10 ml of a 4 per cent w/v solution of *disodium edetate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Using reference solution (e) adjust the attenuation to obtain peaks with a height corresponding to at least 50 per cent of full-scale deflection of the recorder. The test is not valid unless (a) the resolution factor between the first peak (methacycline) and the second peak (6-epidoxycycline) is at least 1.25, (b) the resolution factor between the second peak and the third peak (doxycycline) is at least 2.0 (adjust the content of 2-methylpropan-2-ol in the mobile phase if necessary).

Inject reference solution (a).The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (d) and record the chromatograms. In the chromatogram obtained with the test solution the area of any peak corresponding to methacycline or 6- epidoxycycline is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (2 per cent, with reference to doxycycline hydrochloride), the area of any peak appearing between the solvent peak and the peak corresponding to methacycline and the area of any peak appearing on the tail of the main peak is not greater than 25 per cent of that of the peak corresponding to 6-epidoxycycline in the chromatogram obtained with reference solution (d) (0.5 per cent, with reference to doxycycline hydrochloride).

Loss on drying (2.4.19). Not more than 8.5 per cent, determined on 0.5 g of the contents of the capsules by drying in an oven at 105° for 2 hours.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve the mixed contents of 20 capsules containing about 17.5 mg of anhydrous doxycycline in sufficient *0.01 M hydrochloric acid* to produce 25.0 ml and dilute 4.0 ml of the solution to 25.0 ml with the same solvent.

Reference solution. A 0.0128 per cent w/v solution of *doxycycline hydrochloride IPRS* in *0.01 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
- column temperature: 60°,
- mobile phase: a solution prepared by adding 60 g of *2-methyl-2-propanol* to a volumetric flask with the aid of 200 ml of *water*, adding 400 ml of *phosphate buffer pH 8.0*, 50 ml of a 1 per cent w/v solution of *tetrabutyl ammonium hydrogen sulphate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and 10 ml of a 4 per cent w/v solution of *disodium edetate* previously adjusted to pH 8.0 with *2 M sodium hydroxide* and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation of the area of the peak due to doxycycline is not more than1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}N_2O_8$ in the capsules.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of doxycycline.

Doxycycline Dispersible Tablets

Doxycyline Dispersible Tablets contain doxycycline monohydrate in a suitable dispersible base.

Doxycycline Dispersible Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of doxycycline, $C_{22}H_{24}N_2O_8$.

Usual strength. 100 mg.

Identification

A. Determine by thin - layer chromatography (2.4.17), using the plate coated with *silica gel H*.

Mobile phase. A mixture of 6 volume of *water*, 35 volume of *methanol* and 59 volume of *dichloromethane*.

Test solution. Disperse a quantity of powder containing the equivalent of 0.05 g of anhydrous doxycycline in 100.0 ml of *methanol*, centrifuge and filter the supernatant liquid.

Reference solution (a). A 0.05 per cent w/v solution of *doxycycline hyclate IPRS* in *methanol*.

Reference solution (b). A 0.05 per cent w/v each of *doxycycline hyclate IPRS* and *tetracycline hydrochloride IPRS* in *methanol.*

Spray the plate evenly with a 10.0 per cent w/v solution of *disodium edetate*, adjusted to pH 9.0 with *10M sodium hydroxide*. Allow the plate to dry in a horizontal position for at least 1 hour. Immediately before use dry it at 110° for 1 hour.

Apply to the plate 1 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution is same corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a solution prepared by dissolving 2 g of *sodium chloride* in 7 ml of *hydrochloric acid* and sufficient *water* to produce 1000 ml,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with dissolution medium at the maximum at about 276 nm (2.4.7). Calculate the content of anhydrous doxycycline, $C_{22}H_{24}N_2O_8$ in the medium from the absorbance obtained from a solution of known concentration of *doxycycline hyclate IPRS.*

Q. Not less than 70 per cent of the stated amount of $C_{22}H_{24}N_2O_8$.

Light absorbing impurities (2.4.7). Dissolve a quantity of the powdered tablets in sufficient mixture of 1 volume of *1M hydrochloric acid* and 99 volumes of *methanol* to produce a solution containing the equivalent of 1.0 per cent w/v of anhydrous doxycycline, filter. The absorbance of the filtrate at 490 nm is not more than 0.20, calculated with reference to the dried powdered tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 80 mg of anhydrous doxycycline, in 80 ml of *0.01 M hydrochloric acid,* mix with the aid of ultrasound and dilute with *0.01 M hydrochloric acid* to 100 ml with *0.01M hydrochloric acid*, filter use the supernatant liquid.

Reference solution (a). A 0.08 per cent w/v solution of *doxycycline hyclate IPRS* in the *0.01M hydrochloric acid.*

Reference solution (b). A 0.08 per cent w/v solution of *6-epidoxycycline hydrochloride IPRS* in the *0.01M hydrochloric acid.*

Reference solution (c). A 0.08 per cent w/v solution of *metacycline hydrochloride IPRS* in the *0.01M hydrochloric acid.*

Reference solution (d). A 0.0016 per cent w/v each of *6-epidoxycycline hydrochloride IPRS* and *metacycline hydrochloride IPRS* in the *0.01M hydrochloric acid.*

Reference solution (e). A mixture of 4 volumes of reference solution (a), 1.5 volumes of reference solution (b) and 1 volume of reference solution (c), dilute to 25.0 ml with *0.01 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8µm),
- column temperature: 60°,
- mobile phase: dilute 76 ml of *2-methylpropane-2-ol* in 200 ml of *water* with the aid of ultrasound, add 400 ml of *phosphate buffer pH 8,* 50 ml of 1.0 per cent w/v solution of *tetrabutylammonium hydrogen sulphate*, adjusted to pH 8 with *2M sodium hydroxide* and 10 ml of 4.0 per cent solution of *disodium edetate*, adjusted to pH 8 with *2M sodium hydroxide*, dilute to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (e). The test is not valid unless, the resolution between the peaks corresponding to the metacycline and the 6-epidoxycycline is not less than 1.25 and the resolution between the peaks corresponding to 6-epidoxycycline and doxycycline is not less than 2.0.

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to metacycline and 6-epidoxycycline is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (d) (2.0 per cent) and the area of any other secondary peak is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 1.0 g of powdered tablets by drying in an oven at 60° at pressure not exceeding 2 kPa for 2 hours.

Other test. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), using the chromatographic conditions as described in Related substances.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of anhydrous doxycycline in 20ml of *0.1 M hydrochloric acid* with the aid of ultrasound, dilute to 200.0 ml with *water,* mix. Centrifuge and use the supernatant liquid.

Reference solution. Dissolve 0.115 g of *doxycycline hyclate IPRS* in 20 ml of *0.1 M hydrochloric acid* and dilute to 200.0 ml with *water.*

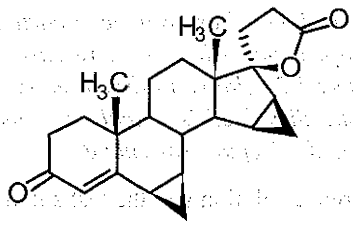
Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}N_2O_8$ in the tablets.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. (1) The label states the quantity of the active ingredient in terms of the equivalent amount of anhydrous doxycycline; (2) The tablets should be dispersed in *water* immediately before use.

Drospirenone



$C_{24}H_{30}O_3$ Mol. Wt. 366.5

Drospirenone is 3-Oxo-6β, 7β: 15β, 16β -dimethylene-17α-pregn-4-en-21, 17-carbolactone.

Drospirenone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{24}H_{30}O_3$, calculated on the dried basis.

Category. Aldosterone receptor antagonist.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *drospirenone IPRS* or with the reference spectrum of drospirenone.

B. Specific optical rotation (see Test).

Tests

Specific optical rotation (2.4.22). –193.0° to –187.0°, determined in a 1.0 per cent w/v solution in *methanol.*

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile.*

Test solution. Dissolve 30 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture. Dissolve the contents of a vial containing 0.06 mg of *drospirenone impurity E IPRS* [3-oxo-6α,7α,15α,16α-tetrahydro-3...H,3 H-dicyclopropa [6,7:15,16]pregn-4-en-21,17-carbolactone *(17-epidrospirenone)] IPRS* in 1.0 ml of the resulting solution.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (c). Dissolve 30 mg of *drospirenone IPRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 35°,
- mobile phase: A. *water,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	63	37
2	63	37
16	52	48
23	52	48
31	20	80
39	20	80
40	63	37
50	63	37

The relative retention time with respect to drospirenone peak for drospirenone impurity E is about 1.1.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to drospirenone impurity E and drospirenone is not less than 5.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{24}H_{30}O_3$.

Storage. Store protected from light and moisture.

Drospirenone and Ethinylestradiol Tablets

Drospirenone and Ethinyl Estradiol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of drospirenone, $C_{24}H_{30}O_3$ and ethinyl estradiol, $C_{20}H_{24}O_2$.

Usual strengths. Drospirenone, 3 mg and Ethinyl Estradiol, 0.03 mg; Drospirenone, 3 mg and Ethinyl Estradiol, 0.02 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

NOTE — A volume of methanol not to exceed 2.0 per cent of the total volume of standard solution may be used to dissolve drospirenone IPRS and ethinyl estradiol IPRS.

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water,*
Speed and time.50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a cellulose filter having an average pore diameter not greater than 0.45 µm, rejecting the first 10 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. Dissolve a suitable quantities of *drospirenone IPRS* and *ethinyl estradiol IPRS* in the *water* to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 6 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 60 volumes of *water* and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- For detection, use UV detector set at 270 nm for drospirenone, in series with a fluorescence detector for ethinyl estradiol, with excitation at 210 nm and detection at 315 nm, or with excitation at 281 nm and detection at 305 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from drospirenone and ethinyl estradiol peaks is not less than 2000 theoretical plates, the tailing factor is not more than 1.5 for drospirenone and ethinyl estradiol and the relative standard deviation for replicate injections for each peak corresponding to drospirenone and ethinyl estradiol is not more than 3.0 per cent.

NOTE — In medium, drospirenone is partially converted into 17-epidrospirenone, which has a relative retention time of approximately 1.2 relative to drospirenone. The amount of drospirenone dissolved is calculated from the sum of drospirenone and 17-epidrospirenone.

Inject the reference solution and the test solution.

Calculate the contents of $C_{24}H_{30}O_3$ and $C_{20}H_{24}O_2$.

Q. Not less than 80 per cent of the stated amount of $C_{24}H_{30}O_3$ and not less than 75 per cent of the stated amount of $C_{20}H_{24}O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Transfer 15 Tablets to a 10-ml glass-stoppered test tube, and add 5.0 ml of mobile phase A, mix with the aid of ultrasound for not less than 5 minutes, and allow to stand in an ice bath for not less 10 minutes. Centrifuge the sample until an almost clear supernatant is obtained. Filter the supernatant, and use the filtrate.

Reference solution (a). A solution containing 0.0045 per cent w/v of *drospirenone IPRS* , 0.00009 per cent w/v of *ethinyl estradiol IPRS* and 0.00009 per cent w/v of Δ 9,11-Ethinyl estradiol.19-Nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol (*ethinyl estradiol impurity B IPRS*) for the tablets containing 0.03 mg of ethinyl estradiol per tablet in mobile

phase A. Or a solution containing 0.0045 per cent w/v of *drospirenone IPRS*, 0.00006 per centw/v of *ethinyl estradiol IPRS* and 0.00006 per cent w/v of Δ 9,11-Ethinyl estradiol.19-Nor-17α-pregna- 1,3,5(10),9 (11)-tetraen-20-yne-3,17-diol (*ethinyl estradiol impurity B IPRS*) for the tablets containing 0.02 mg ofethinyl estradiol per tablet in mobile phase A.

Reference solution (b). Dilute 1 volume of the reference solution (a) to 10 volumes in mobile phase A.

Reference solution (c). Transfer 1.0 ml of 0.054 per cent w/v solution of *drospirenone IPRS* in mobile phase A to a 10-ml volumetric flask, add 1.0 ml of *0.1M hydrochloric acid* heat on water bath for 30 minutes at 40°. Immediately add 1 ml of *0.1M sodium hydroxide* and allow to stand at room temperature. Dilute to 10.0 ml with mobile phase A to obtain a solution containing drospirenone and 17-epidrospirenone.

NOTE— Sodium hydroxide must be added immediately after heating for the reaction to proceed properly. The drospirenone to 17-epidrospirenone ratio must be between 3:1 and 7:1.

Chromatographic system

- a stainless steel column 30 cm x 3.0 mm, packed with octadecylsilane bonded to porous silica (3 µm), in series, by a chromolith column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica,
- column temperature: 40°,
- mobile phase: A.a mixture of 26 volumes of *acetonotrile*, 19 volumes of *methanol* and 55 volumes of *water*,
  B. a mixture of 76 volumes of *acetonotrile*, 19 volumes of *methanol* and 5 volumes of *water*,
- a gradient programme using the conditions given below,
- injection volume: 20 µl.

For detection, use UV detector set at 222 nm, and a fluorescence detector, with excitation at 215 nm, emission at 315 nm. Monitor the signal at 344 nm between 37 and 42 minutes.

NOTE — UV Detector and Fluorescence Detector are connected in series. Use the response at 344 nm to quantify ethinyl estradiol impurity B.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Flow (ml/min.)
0	90	10	0.5
40	90	10	0.5
53	0	100	0.5
59	0	100	1.0
60	90	10	0.5
70	90	10	0.5

Name	Relative retention time	Correction Factor	Limit NMT per cent[a]	Limit NMT per cent[b]	Detector mode
Ethinyl Estradiol Degradation					
6α-hydroxyethinyl estradiol[1]	0.25	1.37	0.3	0.3	FI (215nm/ 315 nm)
6β-hydroxyethinylestradiolis[2]	0.27	1.56	0.3	0.3	FI (215 nm/ 315nm)
6-keto ethinyl estradiol[3]	0.41	0.43	1.5	0.5	UV222 nm
Ethinyl estradiol impurity B[4]	0.88	—	1.0	1.0	FI (215 nm/ 344 nm)
Ethinyl estradiol	1.0	---	---	---	FI (215nm/ 315 nm) and UV 222 nm
Any unspecified degradation product	---	1.0	0.3	0.5	FI(215nm/ 315 nm) and UV 222 nm
Total degradation product	—	---	3.0	2.5	
Drospirenone Degradation Products					
Drospirenone	0.75	—	—	—	UV 222 nm
17-epidrospirenone[5]	0.83	1.0	0.3	0.3	UV 222 nm
Any unspecified degradation product	---	1.0	0.3	0.5	UV 222 nm
Total degradation products	—	---	0.5	1.0	

[a]Limits for drug products labeled to contain 3 mg of drospirenone and 0.03 mg of ethinyl estradiol.

[b]Limits for drug products labeled to contain 3 mg of drospirenone and 0.02 mg of ethinyl estradiol.

[1]19-Nor-6α,17α-pregna-1,3,5(10)-trien-20-yne-3,6,17-triol,

[2]19-Nor-6β,17α-pregna-1,3,5(10)-trien-20-yne-3,6,17-triol,

[3]19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol-6-one,

[4]Δ 9,11-Ethinyl estradiol.19-Nor-17α-pregna-1,3,5(10),9(11)-tetraen-20-yne-3,17-diol,

[5]17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17β-pregn-4-ene-21-carboxylic acid, γ-lactone.

FI = Fluorescence Detector

UV= Ultraviolet Detector

Inject reference solution (a) and test solution.

For Ethinyl estradiol Degradation Products —

In the chromatogram obtained with the test solution, identify the ethnyl estradiol degradation product using the relative retention times given in the table. Calculate the percentage of each ethinyl estradiol degradation product, any unspecified degradation products and ethinyl estradiol related compound B by using their respective corrected peak area and the peak area of ethnyl estradiol from reference solution (a) and compare the limits as shown in the table. Disregard the degradation products less than 0.1 percent.

For Drospirenone Degradation Products —

In the chromatogram obtained with the test solution, identify the drospirenone degradation products using the relative retention times given in the table. Calculate the percentage of each drospirenone degradation product, and any unspecified degradation products by using their respective peak area and the peak area of drospirenone from reference solution (a) and compare the limits as shown in the table. Disregard the degradation products less than 0.1 percent.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Transfer one tablet in 25-ml volumetric flask add 23 ml of mobile phase, mix with the aid of ultrasound for not less than 10 minutes and allow to equilibrate to room temperature dilute to volume with the mobile phase. Centrifuge the sample until an almost clear supernatant is obtained. Use supernatant.

Calculate the contents of $C_{24}H_{30}O_3$ and $C_{20}H_{24}O_2$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay.Determine by liquid chromatography (2.4.14).

Solvent mixture. 10 volumes of solution prepared by dissolving 132 g of *dibasic ammonium phosphate* in 800 ml of *water*, adjusted to pH 6.8 with *orthophosphoric acid* and diluted to 1000 ml and 240 volumes of *water*.

Test solution. Transfer 10 tablets to a 250-ml volumetric flask, add 230 ml of the mobile phase, mixwith the aid of ultrasound for not less than 10 minutes, and allow to equilibrate to room temperature and dilute to volume with the mobile phase. Centrifuge until an almost clear supernatant is obtained .Use the supernatant.

Reference solution.A solution containing 0.012 per cent w/v of *drospirenone IPRS*, and 0.00012 per cent w/v of *ethinyl estradiol IPRS* (for the tablets containing 0.03 mg of ethinyl estradiol per tablet) or 0.00008 per cent w/v of *ethinyl estradiol IPRS* (for the tablets containing 0.02 mg of ethinyl estradiol per tablet) in the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A mixture of equal volumes of *acetonitrile* and the solvent mixture, adjusted to pH 6.8 with *orthophosphoric acid*,
- flow rate: 1.2 ml per minute,
- For detection, use UV detector at 270 nm for drospirenone and a series with a fluorescence detector for ethinyl estradiol, with excitation at 285 nm emission at 315 nm connected in series,
- injection volume: 20 µl.

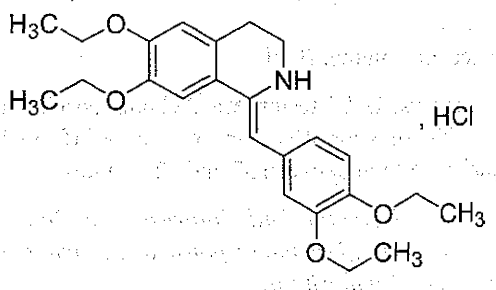
Inject the reference solution. The test is not valid unless the tailing factor for drospirenone and ethinyl estradiol is not more than 1.8 and the relative standard deviation for replicate injections for each of the peaks corresponding to drospirenone and ethinyl estradiol is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the contents of $C_{24}H_{30}O_3$ and $C_{20}H_{24}O_2$ in the tablets using responses of UV detector and flurescence detector.

Storage. Store protected from moisture.

Drotaverine Hydrochloride



$C_{24}H_{31}NO_4,HCl$ Mol.Wt. 434.0

Drotaverine Hydrochloride is (*Z*)-1-(3, 4-Diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride.

Drotaverine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{24}H_{31}NO_4,HCl$, calculated on the dried basis.

Category. Anticholinergic.

Description. Light yellow to yellow with greenish tinge, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained by *drotaverine hydrochloride IPRS* or with the reference spectrum of drotaverine hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 5.0, determined on 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column, 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 48 volumes of *acetonitrile*, 8 volumes of *methanol* and 44 volumes of buffer solution prepared by dissolving 21.8 g of *sodium acetate* in about 500 ml of *water*, add 60 ml of *acetic acid* and dilute to 1000.0 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm)

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in oven at 105° for 3 hours.

Assay. Dissolve 0.4 g in a mixture of 50 ml *glacial acetic acid* and 10 ml of *mercuric acetate solution*. Titrate with *0.1M perchloric acid*, determining the end point potentiometrically (2.4.25) Carry out a blank titration.

1ml of *0.1M perchloric acid* is equivalent to 0.0434 g of $C_{24}H_{31}NO_4.HCl$.

Storage. Store protected from moisture.

Drotaverine Tablets

Drotaverine Hydrochloride Tablets

Drotaverine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of drotaverine hydrochloride, $C_{24}H_{31}NO_4.HCl$.

Usual strengths. 40 mg; 80 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated in the Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 80 mg of Drotaverine Hydrochloride in 50 ml of *methanol* with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.08 per cent w/v solution of *drotaverine hydrochloride IPRS* in *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

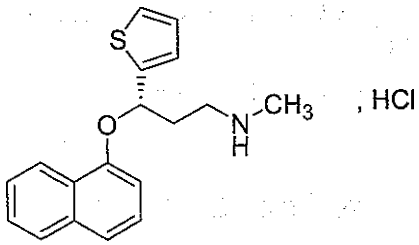
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of buffer solution prepared by dissolving 3.12 g of *sodium dihydrogen orthophosphate* in *water* and dilute to 1000 ml with *water*, adjusted to pH 6.5 with *sodium hydroxide solution*, 40 volumes of *methanol and 35* volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 200 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{31}NO_4.HCl$ in the tablets.

Duloxetine Hydrochloride



$C_{18}H_{19}NOS,HCl$ Mol. Wt. 333.9

Duloxetine Hydrochloride is (*S*)-*N*-Methyl-3-(1-naphthyloxy)-3-(2-thienyl) propan-1-amine hydrochloride.

Duloxetine Hydrochloride contains not less than 97.5 per cent and not more than 102.0 per cent of $C_{18}H_{19}NOS$, HCl, calculated on the dried basis.

Category. Antidepressant.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *duloxetine hydrochloride IPRS* or with reference spectrum of duloxetine hydrochloride.

B. A 0.5 per cent w/v solution gives reaction (A) of chlorides (2.3.1).

C. Enantiomeric purity (see Tests).

Tests

Specific optical rotation (2.4.22). +119.0° to +127.0°, determined in a 1.0 per cent w/v solution in *methanol*, at 20°.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use and perform the test protected from light.

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of *water*.

Test solution (a). Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 200.0 ml with the solvent mixture.

Test solution (b). Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *duloxetine impurity F IPRS((3S)-N-methyl-3-(naphthalene-1-yloxy)-3-(thiophene-3-yl) propane-1-amine)* in the mobile phase. In order to prepare impurity C and D, in situ, heat the solution at 60° for 1 hour.

Reference solution (c). A 0.005 per cent w/v solution of *duloxetine hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3.5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 13 volumes of *acetonitrile*, 17 volumes of *propanol* and 70 volumes of *hexanesulphonate solution* prepared by dissolving 10.3 g of *sodium hexanesulphonate* in a solution prepared by dissolving 1.7 ml *orthophosphoric acid* in 900 ml *water* adjusted to pH 2.5 with *diluted sodium hydroxide solution* and diluted to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Name	Relative retention time
Duloxetine impurity C[1]	0.4
Duloxetine impurity D[2]	0.5
Duloxetine (retention time: about 16 minutes)	1.0
Duloxetine impurityF[3]	1.1

[1]4-[(1*RS*)-3-(methylamino)-1-(thiophen-2-yl)propyl]naphthalen-1-ol,
[2] naphthalen-1-ol,
[3](3*S*)-*N*-methyl-3-(naphthalen-1-yloxy)-3-(thiophen-3-yl)propan-1-amine.

Inject reference solution (b). The test is not valid unless the resolution between peaks due to duloxetine impurity C and D is not less than 1.5 and peak to valley ratio is not less than 4.0.

Inject reference solution (a) and test solution (a). In the chromatogram obtained with test solution (a), the area of any peak corresponding to duloxetine impurity F is not more than 4 times the area of principal peak in the chromatogram obtained with the reference solution (a) (0.4 per cent). The area of any other secondary peak is not more than the area of principal peak in the chromatogram obtained with the reference solution (a) (0.1 per cent). The sum of the areas of all the secondary peaks is not more than 5 times the area of principal peak in the chromatogram obtained with the reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of principal peak in the chromatogram obtained with the reference solution (a) (0.05 per cent).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg each of *duloxetine impurity A IPRS (3R)-N-methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine IPRS)* and the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica gel OD for chiral separation (5 µm),
- column temperature: 40°,
- mobile phase:a mixture of 17 volumes of *isopropanol* and 83 volumes of *hexane,* containing 0.2 per cent v/v of *diethylamine,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

The relative retention time with reference to duloxetine (retention time: about 7 minutes) for duloxetine impurity A is 1.3.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to duloxetine and duloxetine impurity A is not less than 3.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to duloxetine impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14) using the chromatographic system, test solution (b) and reference solution (c) as described under Related substances.

Inject reference solution (c) and test solution (b).

Calculate the content of $C_{18}H_{19}NOS$, HCl.

Storage. Store protected from light and moisture.

Duloxetine Gastro-resistant Tablets

Duloxetine Gastro-resistant Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of duloxetine, $C_{18}H_{19}NOS$.

Usual strengths. 20 mg; 30 mg; 40 mg; 60 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium.750 ml of *0.1 M hydrochloric acid,*
Speed and time.100 rpm and 120 minutes.

Withdraw a suitable volume of the medium, centrifuge at 3500 rpm for 15 minutes, use the supernatant liquid and measure the absorbance of the same, and suitably diluted with the dissolution medium, at the maximum at about 289 nm (2.4.7). Calculate the content of $C_{18}H_{19}NOS$. in the medium from the absorbance obtained from a solution of known concentration of *duloxetine hydrochloride IPRS* in 10 ml *methanol* prepared with the aid of ultrasound and diluted to similar concentration using dissolution medium.

Calculate the content of $C_{18}H_{19}NOS$ in the medium.

Q. Not more than 10 per cent of the stated amount of $C_{18}H_{19}NOS$.

Apparatus No. 1 (Basket),
Medium.1000 ml of *phosphate buffer pH 6.8,*
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium, centrifuge at 3500 rpm for 15 minutes, use the supernatant lquid and measure the absorbance of the same, suitably diluted with the dissolution medium, at the maximum at about 289 nm (2.4.7). Calculate the content of $C_{18}H_{19}NOS$. in the medium from the absorbance obtained from a solution of known concentration of *duloxetine hydrochloride IPRS* in 10 ml *methanol* prepared with the aid of ultrasound and diluted to similar concentration using dissolution medium.

Calculate the content of $C_{18}H_{19}NOS$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{18}H_{19}NOS$.

Other tests. Comply with the tests stated under Tablets (Gastro-resistant tablets).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 60 mg of duloxetine, add 70 ml of the mobile phase, mix with the aid of ultrasound and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.0065 percent w/v solution of *duloxetine hydrochloride IPRS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 3.8 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 5.7 with 10 percent w/v perchloric acid and 60 volumes of *methanol*,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

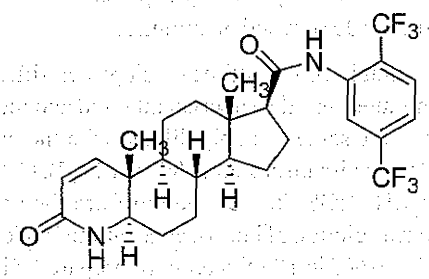
Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{19}NOS$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of duloxetine.

Dutasteride



$C_{27}H_{30}F_6N_2O_2$ Mol. Wt. 528.5

Dutasteride is 3-oxo-*N*-(2,5-bistrifluoromethyl)phenyl-4-aza-5α-androst-1-ene-17β-carboxamide.

Dutasteride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{27}H_{30}F_6N_2O_2$, calculated on the anhydrous basis.

Category. Benign prostatic hypertrophy agent.

Description. A white to off-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dutasteride IPRS* or with the reference spectrum of dutasteride.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *acetonitrile* and 80 volumes of mobile phase B.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution. A 0.001 per cent w/v solution of *dutasteride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 80 volumes of buffer solution prepared by dissolving 3.12 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 ml of *water*, adjusted to pH 5.0 with *dilute sodium hydroxide solution* and 20 volumes of *acetonitrile*,
  B. a mixture of 20 volumes of *water* and 80 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent v/v)
0	60	40
6	60	40
10	50	50
25	40	60
35	40	60
40	30	70
45	60	40
50	60	40

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.25 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *water* and 80 volumes of *acetonitrile.*

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.05 per cent w/v solution of *dutasteride IPRS* in the solvent mixture.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{30}F_6N_2O_2$.

Dutasteride Capsules

Dutasteride Capsules contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dutasteride, $C_{27}H_{30}F_6N_2O_2$.

Usual strength. 0.5 mg.

Identification

In the Assay, the retention time of principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 450 ml of medium A for first 25 minutes followed by addition of 450 ml of medium B,

Speed and time. 50 rpm and 60 minutes.

Medium A. To 1000 ml of *0.1 M hydrochloric acid*, add and dissolve 1.6 g of *pepsin* (label activity 1:3,000).

Medium B. To 1000 ml of *0.1 M hydrochloric acid*, add and dissolve 40 g of *sodium lauryl sulphate.*

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *acetonitrile* and 25 volumes of *water.*

Test solution. Use the filtrate, dilute if necessary, with a mixture of equal volumes of medium A and medium B.

Reference solution. A 0.005 per cent w/v solution of *dutasteride IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with a mixture of equal volumes of medium A and medium B.

Chromatographic system.

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- column temperature:50°,
- mobile phase: A. 0.1 per cent v/v *orthophosphoric acid*, B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 80 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	50	50
14	50	50
15	20	80
20	20	80
22	50	50

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{30}F_6N_2O_2$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{27}H_{30}F_6N_2O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *acetonitrile* and 25 volumes of *water.*

Test solution. Disperse a quantity of the mixed contents of 20 capsules containing 2 mg of Dutasteride in 2 ml of the solvent mixture with the aid of ultrasound for 10 minutes and dilute to 5.0 ml with the solvent mixture, filter.

Reference solution. A 0.0004 per cent w/v solution of *dutasteride IPRS* in the solvent mixture.

Chromatographic system.

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- column temperature: 40°,
- mobile phase: A. *water*, adjusted to pH 2.5 with 10 per cent v/v *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	50	50
55	50	50
57	10	90
62	10	90
65	50	50
70	50	50

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (0.25 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *acetonitrile* and 25 volumes of *water*.

Test solution. Place 5 intact capsules into 50-ml volumetric flask. Disperse in 12.5 ml of *water* with the aid of ultrasound, add 25 ml of *acetonitrile* and again sonicate for 5 minutes and dilute to 50.0 ml with *acetonitrile*, filter.

Reference solution. A 0.005 per cent w/v solution of *dutasteride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: A. 0.1 per cent v/v *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume:20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	45	55
10	45	55
11	20	80
15	20	80
16	45	55
22	45	55

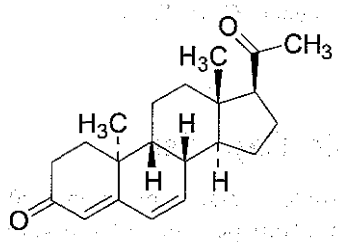
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{30}F_6N_2O_2$ in the capsules.

Storage. Store protected from moisture.

Dydrogesterone



$C_{21}H_{28}O_2$ Mol. Wt. 312.5

Dydrogesterone is 9β,10α-pregna-4,6-diene-3,20-dione.

Dydrogesterone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{28}O_2$ calculated on the dried basis.

Category. Progestogen

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dydrogesterone IPRS* or with the reference spectrum of dydrogesterone.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Specific optical rotation (2.4.22). – 485° to – 469°, determined in a 0.5 per cent w/v solution in *methylene chloride*.

Light absorption (2.4.7). When examined in the range 230 nm to 360 nm, a 0.0015 per cent w/v solution in *methanol* shows

an absorption maximum only at about 286 nm. The ratio of the absorbance at 240 nm to that at 286 nm is not more than 0.12.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Test solution (b). Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 20 mg *dydrogesterone IPRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (c). Dissolve 3 mg of *dydrogesterone impurity A IPRS* in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (d). Dissolve 10 mg of the substance under examination in the 10.0 ml of reference solution (c).

Reference solution (e). Dissolve 10 mg of the substance under examination in the 30 ml of *ethanol (95 per cent)*, add 1 ml of *0.2M sodium hydroxides olution* and heat at 85° for 10 minutes. Cool to room temperature, add 1 ml of *0.24M hydrochloric acid*, add 20 ml of *acetonitrile* and 2 mg of *dydrogesterone impurity B* and dilute to100.0 ml with *water*. This solution contains dydrogesterone and dydrogesterone impurity B and C.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- mobile phase: a mixture of 54 volumes of *water* and 25 volumes of *ethanol (95 per cent)* and 21 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm and at 385 nm,
- injection volume: 10 µl.

Name	Relative retention time
Dydrogesterone impurity A[1] at 385 nm	0.9
Dydrogesterone (Retention time: about 13 minutes)	1.0
Dydrogesterone impurity B[2]	1.1
Dydrogesterone impurity C[3]	1.2

[1] *9β,10α-pregna-4,6,8(14)-triene-3,20-dione,*

[2] pregna-4,6-diene-3,20-dione,

[3] 9β,10α,17α-pregna-4,6-diene-3, 20-dione.

Inject reference solution (d) and (e). The test is not valid unless the resolution between the peaks due to dydrogesterone impurity A and dydrogesterone is not less than 1.1 at 385 nm in the chromatogram obtained with reference solution (d), dydrogesterone impurity B and dydrogesterone is not less than 4.5 and dydrogesterone impurity B and dydrogesterone impurity C is not less than 1.5 in the chromatogram obtained with reference solution (e) at 280 nm.

For impurity A —

Inject reference solution (c) and test solution (a) at 385 nm. Run the chromatogram twice the retention time of the principal peak in test solution (a). The area of any peak corresponding to dydrogesterone impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent).

For impurity B and impurity C —

Inject reference solution (b) and test solution (a) at 280 nm. Run the chromatogram twice the retention time of the principal peak in test solution (a). The area of any peak corresponding to dydrogesterone impurity B is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any peak corresponding to dydrogesterone impurity C is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

- spectrophotometer set at 280 nm,

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the trailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0.

Inject reference solution (a) and test solution (b).

Calculate the content of $C_{21}H_{28}O_2$.

Storage. Store protected from light and moisture.

Dydrogesterone Tablets

Dydrogesterone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of dydrogesterone, $C_{21}H_{28}O_2$.

Usual strength. 10 mg.

Identification

Extract a quantity of the powdered tablets containing 60 mg of Dydrogesterone with 20 ml of *methanol*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dydrogesterone IPRS* or with the reference spectrum of dydrogesterone.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium: 500 ml of 0.3 per cent w/v solution of *sodium lauryl sulphate*,

Speed and time: 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with dissolution medium, if necessary at the maximum at about 295 nm (2.4.7). Calculate the content of $C_{21}H_{28}O_2$ in the medium from the absorbance obtained from a solution of known concentration of *dydrogesterone IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{28}O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Disperse a quantity of powdered tablets containing 50 mg of Dydrogesterone in the mobile phase and dilute to 100.0 ml with the mobile phase.

Test solution (b). Disperse a quantity of powdered tablets containing 20 mg of Dydrogesterone in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 20 mg *dydrogesterone IPRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (c). Dissolve 3 mg of *dydrogesterone impurity A IPRS* in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (d). Disperse a quantity of powdered tablets containing 10 mg of Dydrogesterone in the 10 ml of reference solution (c).

Reference solution (e). Disperse a quantity of powdered tablets containing 10 mg of Dydrogesterone in 30 ml of *ethanol (95 per cent)*, add 1 ml of *0.2M sodium hydroxide solution* and heat at 85° for 10 minutes. Cool to room temperature, add 1 ml of *0.24M hydrochloric acid*, add 20 ml of *acetonitrile* and 2 mg of *dydrogesterone impurity B* and dilute to 100.0 ml with *water*. This solution contains dydrogesterone and dydrogesterone impurity B and C.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (3 µm),
- column temperature 40°,
- mobile phase: a mixture of 54 volumes of *water* and 25 volumes of *ethanol (95 per cent)* and 21 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm and at 385 nm,
- injection volume: 10 µl.

Name	Relative retention time
Dydrogesterone impurity A[1] at 385 nm	0.9
Dydrogesterone (Retention time: about 13 minutes)	1.0
Dydrogesterone impurity B[2]	1.1
Dydrogesterone impurity C[3]	1.2

[1] 9β,10α-pregna-4,6,8(14)-triene-3, 20-dione,

[2] pregna-4,6-diene-3,20-dione,

[3] 9β,10α,17α-pregna-4,6-diene-3, 20-dione.

Inject reference solution (d) and (e). The test is not valid unless the resolution between the peaks due to dydrogesterone impurity A and dydrogesterone is not less than 1.1 at 385 nm in the chromatogram obtained with reference solution (d), dydrogesterone impurity B and dydrogesterone is not less than 4.5 and dydrogesterone impurity B and dydrogesterone impurity C is not less than 1.5 in the chromatogram obtained with reference solution (e) at 280 nm.

For impurity A —

Inject reference solution (c) and test solution (a) at 385 nm. Run the chromatogram twice the retention time of the principal peak in test solution (a). The area of any peak corresponding to dydrogesterone impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent).

For impurity B and impurity C —

Inject reference solution (b) and test solution (a) at 280 nm. Run the chromatogram twice the retention time of the principal peak in test solution (a). The area of any peak corresponding to dydrogesterone impurity B is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any peak corresponding to dydrogesterone impurity C is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following test solution.

Test solution. Disperse one tablet in 10 ml of the mobile phase, with the aid of ultrasound and dilute with the mobile phase to obtain 0.02 per cent w/v solution of Dydrogesterone.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

– spectrophotometer set at 280 nm,

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the trailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0.

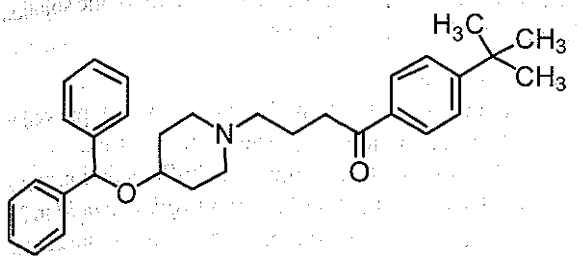
Inject reference solution (a) and test solution (b).

Calculate the content of $C_{21}H_{28}O_2$ in the tablets.

Storage. Store protected from light.

E

Ebastine



$C_{32}H_{39}NO_2$ Mol. Wt. 469.7

Ebastine is 4-(4-Benzhydryloxy-1-piperidyl)-1-(4-*tert*-butylphenyl)butan-1-one.

Ebastine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{32}H_{39}NO_2$, calculated on the anhydrous basis.

Category. Antihistaminic.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ebastine IPRS* or with the reference spectrum of ebastine.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Solution A. A mixture of 65 volumes of *acetonitrile* and 35 volumes of a 0.11 per cent w/v solution of *orthophosphoric acid*, adjusted to pH 5.0 with 4.0 per cent w/v solution of *sodium hydroxide*.

Test solution. Dissolve 0.125 g of the substance under examination in solution A and dilute to 50.0 ml with solution A.

Reference solution (a). A solution containing 0.025 per cent w/v each of *ebastine impurity C IPRS* and *ebastine impurity D IPRS* in solution A. Dilute 1.0 ml of the solution to 100.0 ml with solution A.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with solution A. Dilute 1.0 ml of the solution to 10.0 ml with solution A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with nitrile groups bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a 0.11 per cent w/v solution of *orthophosphoric acid*, adjusted to pH 5.0 with 4.0 per cent w/v solution of *sodium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks corresponding to ebastine impurity D and ebastine impurity C is not less than 2.0.

Name	Relative retention time
Ebastine impurity A[1]	0.04
Ebastine impurity B[2]	0.05
Ebastine impurity D[3]	0.2
Ebastine impurity C[4]	0.22
Ebastine impurity F[5]	0.42
Ebastine impurity G[6]	0.57
Ebastine	1.0
Ebastine impurity E[7]	1.14

[1]benzhydrol,

[2]1-[4-(1,1-dimethylethyl)phenyl]ethanone,

[3]1-[4-(1,1-dimethylethyl)phenyl]-4-(4-hydroxypiperidin-1-yl)butan-1-one,

[4]4-(diphenylmethoxy)piperidine,

[5]1-[4-(1,1-dimethylethyl)phenyl]-4-[*cis*-4-(diphenylmethoxy)-1-oxidopiperidin-1-yl]butan-1-one,

[6]1-[4-(1,1-dimethylethyl)phenyl]-4-[*trans*-4-(diphenylmethoxy)-1-oxidopiperidin-1- yi]butan-1-one,

[7]1-[4-(1,1-dimethylpropyl)phenyl]-4-[4-(diphenylmethoxy)piperidin-1-yl]butan-1-one.

Inject reference solution (b) and the test solution. Run the chromatogram 1.4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphates (2.3.17). Dissolve 2.5 g in 25 ml of *dilute nitric acid*. Boil under a reflux condenser for 10 minutes. Cool and filter. 15 ml of the filtrate complies with the limit test for sulphates (100 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.5 g.

Assay. Dissolve 0.35 g in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04697 g of $C_{32}H_{39}NO_2$.

Storage. Store protected from light.

Ebastine Tablets

Ebastine Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ebastine, $C_{32}H_{39}NO_2$.

Usual strengths. 5 mg; 10 mg; 20 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that of the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 0.5 per cent w/v solution of *sodium lauryl sulphate* in *0.1M hydrochloric acid*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *ebastine IPRS* in *acetonitrile* and dilute with the dissolution medium to obtain a solution having a concentration similar to that of the test solution.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{32}H_{39}NO_2$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{32}H_{39}NO_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 0.05 g of Ebastine in 35 ml of the solvent mixture with the aid of ultrasound and dilute to 50.0 ml with the mobile phase A. Centrifuge and filter.

Reference solution (a). Dilute 0.5 ml of the test solution to a 100.0 ml with the mobile phase A.

Reference solution (b). A solution containing 0.002 per cent w/v each of *ebastine impurity C IPRS* and *ebastine impurity D IPRS* in the solvent mixture. Dilute 2.5 ml of the solution to 25.0 ml with the mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. 40 volumes of buffer solution prepared by dissolving 11.9 g of *orthophosphoric acid* in 25 ml *water* and dilute to 50.0 ml with *water*. Dilute 25.0 ml of the solution to 450.0 ml with *water*, add 6 ml *diethylamine* and dilute to 500 ml with *water*, adjusted to pH 6.0 with *diethylamine* and 60 volumes of *acetonitrile*.
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
40	95	5
55	95	5
57	80	20
65	80	20

Name	Relative retention time
Ebastine impurity C[1]	0.16
Ebastine impurity D[2]	0.17
Ebastine impurity A[3]	0.24
Ebastine impurity B[4]	0.42
Ebastine impurity F[5]	0.5
Ebastine impurity G[6]	0.7
Ebastine (Retention time about 28 minutes)	1.0
Ebastine impurity E[7]	1.35

[1] 4-(diphenylmethoxy)piperidine,

[2] 1-[4-(1,1-dimethylethyl)phenyl]-4-(4-hydroxypiperidin-1-yl)butan-1-one,

[3] benzhydrol,

[4] 1-[4-(1,1-dimethylethyl)phenyl]ethanone,

[5] 1-[4-(1,1-dimethylethyl)phenyl]-4-[*cis*-4-(diphenylmethoxy)-1-oxidopiperidin-1-yl]butan-1-one,

[6] 1-[4-(1,1-dimethylethyl)phenyl]-4-[*trans*-4-(diphenylmethoxy)-1-oxidopiperidin-1- yl]butan-1-one,

[7] 1-[4-(1,1-dimethylpropyl)phenyl]-4-[4-(diphenylmethoxy)piperidin-1-yl]butan-1-one.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to ebastine impurity C

and ebastine impurity D is not less than 1.5 in the chromatogram obtained with reference solution (b) and in the chromatogram obtained with reference solution (a), the tailing factor is not more than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with test solution, the area of any secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent).

Uniformity of content. Complies with the test stated under Tablets as described under Assay with following modifications.

Test solution. Disperse 1 tablet in 1 ml of *water* with intermittent shaking, add 20 ml of the solvent mixture and sonicate for 5 minutes and dilute to 50.0 ml with the solvent mixture, filter.

Reference solution. Dissolve a suitable quantity of *ebastine IPRS* in the solvent mixture and dilute with the solvent mixture in such a manner to get similar concentration to that of test solution.

Calculate the content of $C_{32}H_{39}NO_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of a solution prepared by dissolving 0.3 g of *orthophosphoric acid* in *water* and dilute to 500 ml with *water*, adjusted to pH 5.0 with *1 M sodium hydroxide*, filter and 75 volumes of *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.05 g of Ebastine in 150 ml of the solvent mixture with the aid of ultrasound for 5 minutes and dilute to 250.0 ml with the solvent mixture and filter.

Reference solution. A 0.02 per cent w/v solution of *ebastine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 25 volumes of *0.01 M potassium dihydrogen phosphate* containing 1 ml of *triethylamine* and 75 volumes of *acetonitrile*, adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 255 nm,
- injection volume: 20 µl.

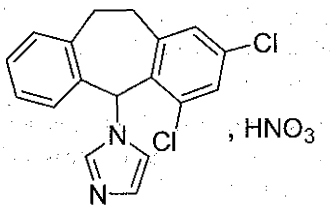
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{32}H_{39}NO_2$ in the tablets.

Storage. Store protected from light and moisture.

Eberconazole Nitrate



$C_{18}H_{14}Cl_2N_2,HNO_3$ Mol. Wt. 392.2

Eberconazole Nitrate is (*RS*)-1-(2,4-Dichloro-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-5-cycloheptenyl)-1*H*-imidazole nitrate.

Eberconazole Nitrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{14}Cl_2N_2.HNO_3$, calculated on the dried basis.

Category. Antifungal.

Description. A white or off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *eberconazole nitrate IPRS* or with the reference spectrum of eberconazole nitrate.

B. It gives reactions for nitrates (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *dimethylformamide* is clear (2.4.1) and not more intensely coloured than reference solution BYS4 (2.4.1).

Specific optical rotation (2.4.22). –0.2° to + 0.2°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 60 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A 0.06 per cent w/v solution of *eberconazole nitrate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v each of *eberconazole nitrate IPRS* and C-transposition compound in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 25 volumes of 0.1 per cent v/v solution of *triethylamine* in *water,* adjusted to pH 8.0 with *orthophosphoric acid* and 75 volumes of *methanol*,
- flow rate: 0.7 ml per minute.
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the eberconazole peak and C-transposition compound is not less than 1.2.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to C-transposition compound is not more than 0.3 per cent and the area of any other secondary peak is not more than 0.1 per cent. The sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Imidazole. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 90 volumes of *toluene*, 10 volumes of *n-propanol* and 0.5 volume of *ammonia.*

Test solution. Dissolve 100 mg of the substance under examination in 5.0 ml of the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *imidazole* in *methanol.*

Apply to the plate 5 µl of the reference solution and 50 µl of the test solution. Allow the mobile phase to rise 15 cm. Dry the plate in air for 15 minutes and expose to iodine vapor for 30 minutes. Any spot corresponding to imidazole in the chromatogram obtained with the test solution is not more intense than the spot obtained with the reference solution (0.1 per cent).

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 60° for 3 hours.

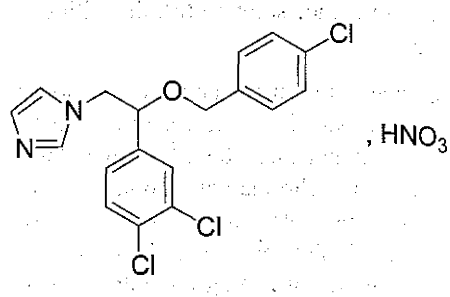
Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{14}Cl_2N_2,HNO_3$.

Storage. Store protected from light.

Econazole Nitrate



$C_{18}H_{15}Cl_3N_2O,HNO_3$ Mol. Wt. 444.7

Econazole Nitrate is (*RS*)-1-[2-[(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate.

Econazole Nitrate contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{18}H_{15}Cl_3N_2O,HNO_3$, calculated on the dried basis.

Category. Antifungal.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *econazole nitrate IPRS* or with the reference spectrum of econazole nitrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution in a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *2-propanol* shows absorption maxima at 265 nm, 271 nm and 280 nm; the ratio of the absorbance at the maximum at 271 nm to that at the maximum at 280 nm is 1.55 to 1.70.

C. It gives reaction (A) of nitrates (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1g of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol.*

Reference solution (a). Dilute 1.0 ml of the test solution to 20.0 ml with *methanol.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 25.0 ml with *methanol.*

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (3 µm),
- column temperature: 35°,
- mobile phase: A. a mixture of 20 volumes of *methanol* and 80 volumes of 0.077 per cent w/v solution of *ammonium acetate*,
  B. a mixture of 40 volumes of *methanol* and 60 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
25	10	90
27	10	90
30	60	40

Name	Relative retention time	Correction factor
Econazole impurity A[1]	0.2	1.4
Econazole impurity B[2]	0.6	—
Econazole (Retention time: about 15 minutes)	1.0	—
Econazole impurity C[3]	1.1	—

[1](1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol,
[2](2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethanamine,
[3]1-(4-chlorobenzyl)-3-[(2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]imidazolium.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to econazole impurities A, B and C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) and the peak corresponding to nitrate ion at the beginning of the chromatogram.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh 0.4 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04447 g of $C_{18}H_{15}Cl_3N_2O,HNO_3$.

Storage. Store protected from light.

Econazole Cream

Econazole Nitrate Cream

Econazole Cream contains Econazole Nitrate in a suitable cream base.

Econazole Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of econazole nitrate, $C_{18}H_{15}Cl_3N_2O, HNO_3$.

Usual strength. 1 per cent w/w.

Identification

A. Mix a quantity of the cream containing 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of *1M sulphuric acid* and 4 volumes of *methanol* and shake with two 50 ml quantities of *carbon tetrachloride* discarding the organic layers. Make the aqueous phase alkaline with *2 M ammonia* and extract with two 40 ml quantities of *chloroform*. Combine the *chloroform* extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and dilute the filtrate to 100 ml with *chloroform*. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of *0.1M hydrochloric acid* and 9 volumes of *propan-2-ol*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at 265 nm, 271 nm and 280 nm. The ratio of the absorbance at 271 nm to that at about 280 nm is 1.55 to 1.77.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the econazole peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 2.5 g of *potassium dihydrogen orthophosphate* and 2.5 g of *dipotassium hydrogen orthophosphate* in 1000 ml of *water*.

Internal standard solution. A 0.05 per cent w/v solution of *miconazole nitrate IPRS* in *methanol.*

Test solution (a). Mix a quantity of the cream containing 10 mg of Econazole Nitrate with 20 ml of the internal standard solution and 55 ml of *methanol.* Warm on a water-bath for 30 seconds, shake for 1 minute, repeat the process twice and add 25 ml of the buffer solution. Cool in an ice bath for 15 minutes, centrifuge for 10 minutes and use the supernatant liquid, filtered if necessary.

Test solution (b). Prepare in the same manner as test solution (a) but using 20 ml of *methanol* in place of the internal standard solution.

Reference solution. A 0.1per cent w/v solution of *econazole nitrate IPRS* in *methanol.* Transfer 10.0 ml of the solution to 100-ml volumetric flask, add 20 ml of the internal standard solution, 45 ml of *methanol* and 25 ml of the buffer solution.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of 25 volumes of the buffer solution and 75 volumes of *methanol,*
- flow rate: 2 ml per minute,
- spectrophotometer set at 232 nm,
- injection volume: 20 µl.

Inject the reference solution and test solution (a) and (b).

Calculate the content of $C_{18}H_{15}Cl_3N_2O, HNO_3$ in test solution (a) in the cream.

Storage. Store protected from light at a temperature not exceeding 30°. If it is packed in aluminium tubes the inner surfaces of the tubes should be coated with a suitable lacquer.

Econazole Pessaries

Econazole Nitrate Pessaries; Econazole Vaginal Tablets

Econazole Pessaries contain Econazole Nitrate in a suitable base.

Econazole Pessaries contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of econazole nitrate, $C_{18}H_{15}Cl_3N_2O,HNO_3$.

Usual strength. 150 mg.

Identification

A. Mix a quantity of the crushed pessaries containing 40 mg of Econazole Nitrate with 20 ml of a mixture of 1 volume of *1 M sulphuric acid* and 4 volumes of *methanol* and shake with two quantities, each of 50 ml, of *carbon tetrachloride,* discarding the organic layers. Make the aqueous phase alkaline with *2 M ammonia* and extract with two quantities, each of 40 ml, of *chloroform.* Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate,* filter and dilute the filtrate to 100 ml with *chloroform.* Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 1 volume of *0.1 M hydrochloric acid* and 9 volumes of *2-propanol.*

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at 265 nm, 271 nm and 280 nm. The ratio of the absorbance at about 271 nm to that at 280 nm is 1.55 to 1.70.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), using a precoated silica gel plate (such as Merck silica gel 60 plates).

Mobile phase. A mixture of 70 volumes of *chloroform,* 20 volumes of *methanol* and 10 volumes of an 85 per cent w/v solution of *formic acid.*

Test solution. Mix a quantity of the crushed pessaries containing 40 mg of Econazole Nitrate with 40 ml of *methanol* and heat under a reflux condenser for 15 minutes. Allow to cool, filter, wash the filter paper with *methanol* and evaporate the filtrate and washings to a volume of about 5 ml. Filter through a filter paper (such as Whatman No. 50 paper), wash the paper with *methanol,* evaporate the filtrate and washings to dryness and dissolve the residue in 2 ml of *methanol.*

Reference solution (a). Dilute 0.5 ml of the test solution to 100.0 ml with *methanol.*

Reference solution (b). A 2.0 per cent w/v solution of *econazole nitrate IPRS* in *methanol.*

Apply to the plate 20 µl of each solution. After development, dry the plate in air and expose to iodine vapour for 1 hour. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot with an R_f value higher than 0.9.

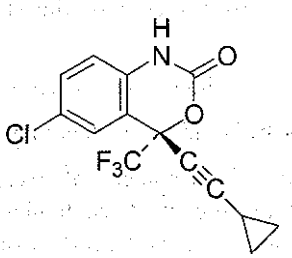
Other tests. Comply with the tests stated under Pessaries.

Assay. Dissolve 5 pessaries in 250.0 ml of *anhydrous glacial acetic acid* with the aid of gentle heat and allow to cool. Titrate 100.0 ml of the solution with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04447 g of $C_{18}H_{15}Cl_3N_2O,HNO_3$.

Storage. Store protected from light.

Efavirenz



$C_{14}H_9ClF_3NO_2$ Mol. Wt. 315.7

Efavirenz is (4*S*)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazin-2-one.

Efavirenz contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_9ClF_3NO_2$, calculated on the dried basis.

Category. Antiretroviral.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *efavirenz IPRS* or with the reference spectrum of efavirenz.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). −100.0° to −90.0°, determined in a 0.3 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *methanol*.

Reference solution. Dilute 1.0 ml of the test solution to 10.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 3.0 ± 0.05 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. Determine the amount of related substances by area normalisation method. The content of any individual impurity is not more than 0.5 per cent and the sum of all the impurities is not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying at 105° in an oven for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.12 g of the substance under examination in 100.0 ml of *methanol*.

Reference solution. A 0.12 per cent w/v solution of *efavirenz IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 3.0 ± 0.05 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_9ClF_3NO_2$.

Storage. Store protected from light.

Efavirenz Capsules

Efavirenz Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of efavirenz, $C_{14}H_9ClF_3NO_2$.

Usual strengths. 50 mg; 100 mg; 200 mg.

Identification

A. When examined in the range 220 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum as obtained with *efavirenz IPRS* of the same concentration.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix well the contents of 20 capsules and disperse a quantity of the mixed contents containing 50 mg of Efavirenz in *methanol* and dilute to 50.0 ml with *methanol.*

Reference solution. A 0.1 per cent w/v solution of *efavirenz IPRS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 3.0 ± 0.05 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. Determine the amount of related substances by the area normalisation method. The content of any individual impurity is not more than 1.0 per cent and the sum of all impurities is not more than 2.0 per cent.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a 1 per cent w/v solution of *sodium lauryl sulphate,*

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate and dilute a suitable volume of the filtrate if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 247 nm (2.4.7). Calculate the content of efavirenz, $C_{14}H_9ClF_3NO_2$ in the medium from the absorbance obtained from a solution of known concentration of *efavirenz IPRS* in the same solvent.

Q. Not less than 70 per cent of the stated amount of $C_{14}H_9ClF_3NO_2$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix well the contents of 20 capsules and shake a quantity of the mixed contents containing about 60 mg of Efavirenz with sufficient *methanol* to obtain a solution containing 6 mg of Efavirenz per ml. Disperse the mixture with the aid of ultrasound for 20 minutes, filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate, and dilute 10.0 ml of the filtrate to 50.0 ml with *methanol.*

Reference solution. A 0.12 per cent w/v solution of *efavirenz IPRS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilyl silica gel (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 3.0 ± 0.05 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_9ClF_3NO_2$.

Storage. Store protected from light.

Efavirenz Tablets

Efavirenz Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of efavirenz, $C_{14}H_9ClF_3NO_2$.

Usual strength. 600 mg.

Identification

A. When examined in the range 220 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum as obtained with *efavirenz IPRS* of the same concentration.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 1000 ml of a 2 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, reject the first few ml of the filtrate and dilute a suitable volume of the filtrate if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 247 nm (2.4.7). Calculate the content of efavirenz, $C_{14}H_9ClF_3NO_2$ in the medium from the absorbance obtained from a solution of known concentration of *efavirenz IPRS* in the same solvent.

Q. Not less than 70 per cent of the stated amount of $C_{14}H_9ClF_3NO_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets with a suitable quantity of *methanol* to obtain a mixture containing 0.1 per cent w/v of Efavirenz and filter through a membrane filter disc with an average pore diameter not exceeding 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution. A 0.1 per cent w/v solution of *efavirenz IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 3.0 ± 0.05 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any individual impurity is not more than 1.0 per cent and the sum of areas of all impurities is not more than 2.0 per cent, calculated by area normalisation.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 120 mg of Efavirenz in *methanol* with the aid of ultrasound for 20 minutes and dilute to 100.0 ml with *methanol*, filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm.

Reference solution. A 0.12 per cent w/v solution of *efavirenz IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.86 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 3.0 ± 0.05 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 252 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the efavirenz peak is not less than 6000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_9ClF_3NO_2$.

Storage. Store protected from light.

Efavirenz, Emtricitabine and Tenofovir Tablets

Efavirenz,Emtricitabine and Tenofovir Disoproxil Fumarate Tablets.

Efavirenz, Emtricitabine and Tenofovir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of efavirenz, $C_{14}H_9ClF_3NO_2$, emtricitabine, $C_8H_{10}FN_3O_3S$ and tenofovir disoproxil fumarate, $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$.

Usual strength. 600 mg Efavirenz, 200 mg Emtricitabine and 300 mg Tenofovir Disoproxil Fumarate.

Identification

A. In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *methyl isobutyl ketone*, 25 volumes of *glacial acetic acid* and 25 volumes of *water* in a separating flask. Mix thoroughly and allow the layers to separate. Use the upper layer.

Test solution. Shake a quantity of the powdered tablets containing about 20 mg of Tenofovir Disoproxil Fumarate, add about 10 ml of *methanol*, sonicate for 10 minutes and dilute to 20.0 ml with *methanol*, filter.

Reference solution (a). A 0.065 per cent w/v solution of *emtricitabine IPRS* in *methanol*.

Reference solution (b). A 0.1 per cent w/v solution of *tenofovir disoproxil fumarate IPRS* in *methanol*.

Reference solution (c). A 0.2 per cent w/v solution of *efavirenz IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spots in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solution (a), (b) and (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 1000 ml of 2.0 per cent w/v *sodium lauryl sulphate*,
Speed and time. 100 rpm and 45 minutes.
Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution containing 0.06 per cent w/v of *efavirenz IPRS*, 0.02 per cent w/v of *emtricitabine IPRS* and 0.03 per cent w/v of *tenofovir disoproxil fumarate IPRS* dissolved in minimum amount of *methanol* and diluted with the dissolution medium.

Chromatographic system
- a stainless steel column 5 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3 µm) (Such as Inertsil C8),
- column temperature: 40°,
- mobile phase: A. a buffer solution prepared by dissolving *0.01 M monobasic potassium phosphate*, adjusted to pH 3.0 with *orthophosphoric acid*,
  B. a mixture of 20 volumes of buffer solution and 80 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	94	6
3	94	6
5	50	50
13	20	80
14	94	6
17	94	6

The retention time of emtricitabine is about 2 minutes, tenofovir disoproxil is about 6 minutes and efavirenz is about 10 minutes.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_9ClF_3NO_2$, $C_8H_{10}FN_3O_3S$ and $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{14}H_9ClF_3NO_2$, $C_8H_{10}FN_3O_3S$ and $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Store crushed powder of tablets in amber colored glass bottle with appropriate lids.

For emtricitabine—

Test solution. Dissolve a quantity of the powdered tablets containing about 50 mg of Emtricitabine in 5 ml of *methanol*, add 30 ml of mobile phase A and dilute to 50.0 ml with mobile phase A, filter.

Reference solution. A 0.001 per cent w/v solution of *emtricitabine IPRS* in mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Supelco HS Discovery C18),
- mobile phase: A. a mixture of 95 volumes of buffer solution prepared by dissolving 1.9 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 3.8 with *glacial acetic acid* and 5 volumes of *methanol*,
  B. a mixture of 30 volumes of buffer solution and 70 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate:1 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
30	100	0
35	0	100
55	0	100
60	100	0
75	100	0

The relative retention time with reference to emtricitabine for (2*R*,5*R*)-5-(4-amino-5-fluoro-2-oxo-2*H*-pyrimidin-1-yl)-(1,3)-oxathiolan-2-carboxylic acid (emtricitabine impurity A) is about 0.25, for 2,3'dideoxy-5-fluoro-3'-thiouridine (emtricitabine impurity B) is about 1.15 and for 4-amino-5-fluoro [2-hydroxymethyl-3-oxo-1,3oxathilane- 5yl]pyrimidine-2(1*H*)-one (emtricitabine impurity C) is about 0.32.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to emtricitabine impurities A and C is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent), the area of any peak due to emtricitabine impurity B is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (3.0 per cent).

For tenofovir disoproxil fumarate —

Test solution. Disperse a quantity of the powdered tablets containing about 50 mg of Tenofovir Disoproxil Fumarate in 35 ml of *methanol* with the aid of ultrasound for 15 minutes and dilute to 50.0 ml with *methanol*, filter.

Reference solution (a). A 0.001 per cent w/v solution of *tenofovir disoproxil fumarate IPRS* in *methanol*.

Reference solution (b). A 0.02 per cent w/v solution of *fumaric acid* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil C18),
- mobile phase: A. dissolve 1.9 g of *ammonium acetate* in 1000 ml of *water* and adjusted to pH 3.8 with *glacial acetic acid*,

  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 25 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
10	50	50
25	50	50
50	20	80
60	95	5
75	95	5

The relative retention time with reference to tenofovir disoproxil fumarate for tenofovir impurity A (2[(isopropoxycarbonyl)oxy]methyl hydrogen {[(1*R*)-2-(6-amino-9*H*-purin-9-yl)-1-methylethoxy]methyl} phosphonate) is about 0.48, for tenofovir impurity B (9-[(*R*)-[(2-[isopropoxy]-2-[((isopropoxycarbonyl)oxy) methoxy]phosphinyl)methoxy] propyl]adenine) is about 0.79, for tenofovir impurity C (9-[2-[[bis[[Isopropoxy carbonyl)oxy]methoxy]phosphinyl] methoxy]ethyl]adenine fumarate) is about 0.87 and for specified unidentified impurity is about 1.05.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to tenofovir impurity A, multiplied with the correction factor 0.63 is not more than 2.0 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). Ignore any peak due to fumaric acid.

For efavirenz —

Solvent mixture. 50 volumes of *water* and 50 volumes of *acetonitrile*.

Test solution. Dissolve a quantity of the powdered tablets containing about 100 mg of Efavirenz in 70 ml of the solvent mixture and dilute to 100.0 ml with the same solvent, filter.

Reference solution. A 0.001 per cent w/v solution of *efavirenz IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C18),

– mobile phase: A. a mixture of 50 volumes of a buffer solution prepared by dissolving 8.6 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 50 volumes of *acetonitrile*

B. a mixture of 25 volumes of buffer solution and 75 volumes of *acetonitrile*,
– a gradient programme using the conditions given below,
– flow rate: 1.5 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 20 µl

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
15	100	0
25	0	100
35	0	100
37	100	0
45	100	0

The relative retention time with reference to efavirenz for (*S*)-5-chloro-α-(cyclopropylethynyl)-2-amino-α-(trifluoromethyl)-benznemethanol (efavirenz impurity A) is about 0.82.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to efavirenz impurity A is not more than 0.5 per cent. The area of any other secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 3.0 per cent. Ignore any peak at relative retention time of about 0.56.

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of *water* and 70 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity containing about 200 mg of Emtricitabine in *water* with the aid of ultrasound, warm at 70° for 10 minutes. Add about 35 ml of *methanol*, sonicate for 30 minutes with intermittent swirling and dilute to 100 ml with *methanol*, filter. Dilute 5.0 ml of the solution to 200.0 ml with the solvent mixture.

Reference solution. A solution containing 0.025 per cent w/v of *emtricitabine IPRS*, 0.04 per cent w/v of *tenofovir disoproxil fumarate IPRS* and 0.08 per cent w/v of *efavirenz IPRS* in *methanol*. Dilute 5.0 ml of the solution to 25.0 ml with the solvent mixture.

Chromatographic system
– a stainless steel column 5 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3µm) (Such as Inertsil C8),
– column temperature. 40°,
– mobile phase: A. a *0.01 M monobasic potassium phosphate*, adjusted to pH 3.0 with *orthophosphoric acid*,

B. a mixture of 20 volumes of mobile phase A and 80 volumes of *acetonitrile*,
– a gradient programme using the conditions given below,
– flow rate: 1.5 ml per minute,
– spectrophotometer set at 250 nm,
– injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	94	6
3	94	6
5	50	50
13	20	80
14	94	6
17	94	6

The retention time of tenofovir disoproxil is about 6 minutes, emtricitabine is about 2 minutes and efavirenz is about 10 minutes.

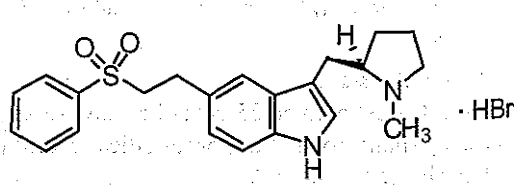
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 for each component and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_9ClF_3NO_2$, $C_8H_{10}FN_3O_3S$ and $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Eletriptan Hydrobromide



$C_{22}H_{26}N_2O_2S,HBr$ Mol wt. 463.4

Eletriptan Hydrobromide is (*R*)-3-((1-Methyl-2-pyrrolidinyl) methyl)-5-(2-(phenylsulfonyl)ethyl)-1*H*-indole hydrobromide.

Eletriptan Hydrobromide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{26}N_2O_2S,HBr$, calculated on the dried basis.

Category. Antimigraine.

Description. A cream to pale brown powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *eletriptan hydrobromide IPRS* or with the reference spectrum of eletriptan hydrobromide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.24). +4.5 to +8.5, determined on 0.5 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.00015 per cent w/v solution of *eletriptan hydrobromide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: A. dissolve 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, add 2.0 ml of *triethylamine*, adjusted to pH 3.5 with *orthophosphoric acid*,
  B. a mixture of 80 volumes *acetonitrile* and 20 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0.01	80	20
25	50	50
33	05	95
38	05	95
42	80	20
50	80	20

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.3 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Bromide. 16.0 per cent to18.0 per cent.

Dissolve 0.15 g in 100.0 ml of *water*. Titrate with *0.1 M silver nitrate*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.00799 g of bromide.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

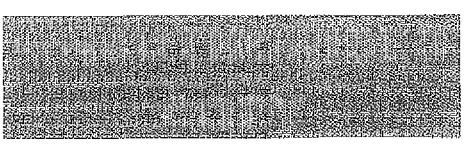
Test solution. Dissolve 25 mg of the substance under examination in 50.0 ml of solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *eletriptan hydrobromide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, add 2.0 ml of *triethylamine*, adjusted to pH 3.5 with *orthophosphoric acid* ,
  B. a mixture of 80 volumes *acetonitrile* and 20 volumes of *water*,
- a gradient programme using the conditions given below
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0.01	75	25
16	50	50
20	05	95
25	05	95
28	75	25
35	75	25



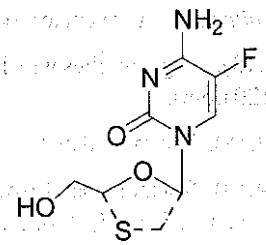
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{26}N_2O_2S,HBr$.

Storage. Store protected from moisture.

Emtricitabine



$C_8H_{10}FN_3O_3S$ Mol. Wt. 247.3

Emtricitabine is 4-amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2-(1*H*)-pyrimidone.

Emtricitabine contain not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_{10}FN_3O_3S$, calculated on the dried basis.

Category. Antiretroviral.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *emtricitabine IPRS* or with the reference spectrum of emtricitabine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). −115.0° to −105.0°, determined in a 0.25 per cent w/v solution in *water*.

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 25 mg of the substance under examination in 50 ml of the mobile phase.

Reference solution. Dissolve 25 mg of *racemic emtricitabine IPRS* in 25.0 ml of the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with a chiral stationary phase (5 µm) (Such as Chirobiotic V),
- mobile phase: a mixture of 1000 volumes of *methanol*, 2 volumes of *diethyl amine* and 1volume of *glacial acetic acid*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume: 10 µl.

Inject the reference solution. The elution order is, the 5-fluoro-1-(2*R*,5*S*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine isomer followed by the other isomer. The resolution between the two isomers should not be less than 2.0.

Inject the test solution and measure the areas of the two isomers.

Calculate the content of the 5-fluoro-1-(2*S*,5*R*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine isomer by area normalization, not more than 0.3 per cent.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 25 mg of the substance under examination in 25 ml of the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *emtricitabine IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with pentaflurophenyl bonded to silica (5 µm) (Such as F-5 Supelco discovery),
- mobile phase: a mixture of 99 volumes of *0.025 M ammonium acetate solution* and adjusted to pH 5.0 with *glacial acetic acid*, and 1 volume of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency in not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and test solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak due to reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak due to reference solution (b) (2.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 25 mg of the substance under examination in 25.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *emtricitabine IPRS* in the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with pentaflurophenyl bonded to silica (5 µm) (Such as F-5 Supelco discovery),
- mobile phase: a mixture of 95 volumes of *0.025 M ammonium acetate solution* adjusted the pH to 5.0 and 5 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency in not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{10}FN_3O_3S$.

Storage. Store protected from light and moisture.

Emtricitabine Capsules

Emtricitabine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of emtricitabine, $C_8H_{10}FN_3O_3S$.

Usual strength. 200 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Disperse the quantity of contents of the capsules containing about 1 mg of Emtricitabine in 100 ml of *methanol* and filter.

When examined in the range 200 nm to 400 nm (2.4.7), the filtrate shows absorption maxima at the same wavelengths as 0.001 per cent w/v solution of *emtricitabine IPRS* in *methanol*.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not more than 0.5 µm, rejecting the first 2 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. Dissolve 27.5 mg of *emtricitabine IPRS* in 15 ml of *methanol*, dilute to 25 ml with the mobile phase. Dilute 2.0 ml of the solution to 10.0 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the reference solution and the test solution.

Q. Not less than 75 per cent of the stated amount of $C_8H_{10}FN_3O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the contents of the capsules containing 100 mg of Emtricitabine, disperse in 100.0 ml of the mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of *emtricitabine IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with pentaflurophenyl bonded to silica (5 µm) (Such as F-5 Supelco discovery),
- mobile phase: a mixture of 99 volumes of a buffer solution prepared by dissolving 1.9 g of *ammonium acetate* in 1000 ml of *water* and adjusted to pH 5.0 with *glacial acetic acid*, and 1 volume of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the column efficiency in not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak due to the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak due to the reference solution (b) (3.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing about 100 mg of Emtricitabine, disperse

in 100.0 ml of *methanol* and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.1 percent w/v solution of *emtricitabine IPRS* in *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Intersil ODS 3V),
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 1.9 g of *ammonium acetate* in 1000 ml of *water* and adjusted to pH 3.8 with *glacial acetic acid*, and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 277 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency in not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{10}FN_3O_3S$ in the capsules.

Storage. Store protected from moisture.

Emulsifying Wax

Anionic Emulsifying Wax

Emulsifying Wax is a waxy solid containing 90 parts of Cetostearyl Alcohol, 10 parts of Sodium Lauryl Sulphate or sodium salts of similar sulphated higher primary aliphatic alcohols, and 4 parts of Purified Water.

Category. Pharmaceutical aid (emulsifying agent).

Description. An almost white or pale yellow, waxy solid or flakes. It becomes soft on warming.

Identification

The residue obtained in the test for Unsaponifiable matter melts at about 52° (2.4.21).

Tests

Acidity. Weigh 20.0 g, add a mixture of 40 ml of *ether* and 75 ml of *ethanol (95 per cent)*, previously neutralised to *phenolphthalein solution*, and warm gently until solution is effected. Titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator until a pink colour which persists for at least 15 seconds is obtained. Not more than 1.0 ml of *0.1 M sodium hydroxide* is required.

Alkalinity. 25 ml of a 20 per cent w/v dispersion in warm *ethanol (95 per cent)*, previously neutralised to *phenolphthalein solution* and cooled, exhibits no colour on the addition of 0.5 ml of *phenolphthalein solution*.

Saponification value (2.3.37). Not more than 2.0, determined on 20.0 g.

Unsaponifiable matter (2.3.39). Not less than 86.0 per cent, calculated on the anhydrous basis, determined on 5 g and omitting the titration of the residue.

Iodine value (2.3.28). Not more than 3.0, determined by the iodine monochloride method.

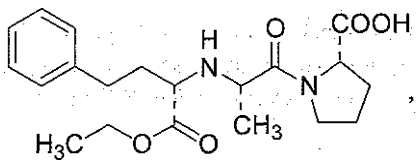
Alcohols. To 3.5 g of the residue obtained in the test for Unsaponifiable matter add 12 g of *stearic anhydride* and 10 ml of *xylene* and heat gently under a reflux condenser for 30 minutes. Cool, add a mixture of 40 ml of *pyridine* and 4 ml of *water*, reflux for a further 30 minutes and titrate the hot solution with *1 M sodium hydroxide* using *phenolphthalein solution* as indicator. Repeat the operation omitting the residue. The difference between the titrations is not less than 12.8 ml and not more than 14.2 ml.

Sodium alkyl sulphates. Not less than 8.7 per cent, calculated as $C_{12}H_{25}O_4SNa$, on the anhydrous basis, determined by the following method. Weigh 0.25 g, dissolve as completely as possible in 15 ml of *chloroform*, add 30 ml of *water*, 10 ml of *1 M sulphuric acid* and 1 ml of *dimethyl yellow-oracet blue B solution* and titrate with *0.004 M benzethonium chloride*, shaking vigorously and allowing the layers to separate after each addition, until the chloroform layer acquires a permanent clear green colour.

1 ml of *0.004 M benzethonium chloride* is equivalent to 0.001154 g of $C_{12}H_{25}O_4SNa$.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.6 g.

Enalapril Maleate



$C_{20}H_{28}N_2O_5,C_4H_4O_4$ Mol. Wt. 492.5

Enalapril Maleate is (2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]pyrrolidin-2-carboxylic acid hydrogen maleate.

Enalapril Maleate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{28}N_2O_5,C_4H_4O_4$, calculated on the dried basis.

Category. Antihypertensive.

Description. An off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *enalapril maleate IPRS* or with the reference spectrum of enalapril maleate.

B. Melts at about 144° (2.4.21).

Tests

Specific optical rotation (2.4.22). −43.5° to −41.0°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 50 volumes of *acetonitrile* and 950 volumes of buffer solution prepared by dissolving 2.8 g of *sodium dihydrogen phosphate monohydrate* in 950 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Test solution. Dissolve 30 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). A 0.0003 per cent w/v solution of *enalapril impurity A ((2S)-1-[(2S)-2-[[(1R)-1-(ethoxycarbonyl)-3- phenylpropyl] amino]propanoyl] pyrrolidine-2-carboxylic acid)IPRS* in the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.1 mm, packed with styrene-divinylbenzene copolymer (5 µm),
- column temperature: 70°,
- mobile phase: A. a mixture of 5 volumes of *acetonitrile* and 95 volumes of buffer solution pH 6.8 prepared by dissolving 2.8 g of *sodium dihydrogen phosphate monohydrate* in 950 ml of *water*. Adjusted to pH 6.8 with *dilute sodium hydroxide solution* and dilute to 1000 ml with *water*,

  B. a mixture of 34 volumes of buffer solution pH 6.8 and 66 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
20	40	60
25	40	60
26	95	5
30	95	5

The retention time of enalapril peak is about 11 minutes and of enalapril impurity A is about 12 minutes.

Inject reference solution (b). The test is not valid unless the peak–to–valley ratio is not less than 10, where H_p is the height above the baseline of the peak due to enalapril impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to enalapril.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to enalapril impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of any other secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of areas of all the secondary peaks other than the peak due to enalapril impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent) and the peak due to maleic acid.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 2 hours.

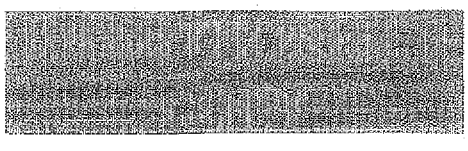
Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 30 mg of the substance under examination and dissolve in 100.0 ml of the mobile phase.

Reference solution. A freshly prepared 0.03 per cent w/v solution of *enalapril maleate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with a rigid spherical styrene divinylbenzene copolymer (5 to 10 µm),
- mobile phase: a mixture of 4 volumes of *mixed phosphate buffer pH 6.8* and 1 volume of *acetonitrile*,



- column temperature :70°,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 300 theoretical plates and the relative standard deviation for the area of the peak due to enalapril maleate is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$.

Storage. Store protected from light.

Enalapril Maleate Tablets

Enalapril Maleate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of enalapril maleate, $C_{20}H_{28}N_2O_5,C_4H_4O_4$.

Usual strengths. 2.5 mg; 5 mg; 10 mg; 20 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve an accurately weighted quantity of *enalapril maleate IPRS* in the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 75 volumes of buffer solution prepared by dissolving 1.38 g of *monobasic sodium phosphate* in 800 ml of *water* adjusted to pH 2.2 with *orthophosphoric acid* and dilute to 1000 ml with *water* and 25 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{20}H_{28}N_2O_5,C_4H_4O_4$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Disperse 1 tablet in *phosphate buffer pH 2.0* with the aid of ultrasound and dilute to obtain a concentration of 0.01 per cent w/v solution of Enalapril Maleate.

Reference solution. A 0.01 per cent w/v solution of *enalapril maleate IPRS* in the same buffer solution.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a filtered and degassed mixture of 75 volumes of *phosphate buffer pH 2.0* and 25 volumes of *acetonitrile*,
- column temperature 50°,
- flow rate: 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Enalapril Maleate, add 150 ml of *phosphate buffer pH 2.0*, disperse with the aid of ultrasound for 15 minutes, shake for another 30 minutes and dilute with the buffer to 250.0 ml, mix and filter.

Reference solution. A 0.02 per cent w/v solution of *enalapril maleate IPRS* in *phosphate buffer pH 2.0*.

Follow the chromatographic procedure described under Uniformity of content.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in the tablets.

Enalapril Maleate and Hydrochlorothiazide Tablets

Enalapril Maleate and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of enalapril maleate $C_{20}H_{28}N_2O_5,C_4H_4O_4$ and hydrochlorothiazide $C_7H_8ClN_3O_4S_2$.

Usual strengths. Enalapril Maleate, 2.5 mg and Hydrochlorothiazide, 12.5 mg; Enalapril Maleate, 5 mg and Hydrochlorothiazide, 12.5 mg; Enalapril Maleate, 10 mg and Hydrochlorothiazide, 25 mg.

Identification

A. In the Assay of enalapril maleate, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. In the Assay of hydrochlorothiazide, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm, 30 minutes.

Withdraw a suitable volume of the medium and filter.

For enalapril maleate — Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as obtained above.

Reference solution. Dissolve a quantity of *enalapril maleate IPRS* with the dissolution medium to obtain a solution having a similar concentration to the test solution.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octylsilane bonded to porous silica (10 µm),
- column temperature: 80°,
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 68 volumes of *water* and 2 volumes of a buffer solution prepared by dissolving 136 g of *monobasic potassium phosphate* in 800 ml *water*, adjusted to pH 4.0 with *orthophosphoric acid* and dilute to 1000 ml,
- flow rate: 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$.

For hydrochlorothiazide — Measure the difference in the absorbance of the filtrate, suitably diluted if necessary with the dissolution medium at the maximum at about 320 nm and 360 nm (2.4.7). Calculate the content of $C_7H_8ClN_3O_4S_2$ in the medium from the absorbance, similarly determined, obtained from a solution of *hydrochlorothiazide IPRS* prepared by dissolving in minimum quantity of *methanol* and diluted suitably with the dissolution medium to get similar concentration to the test solution.

Calculate the content of $C_7H_8ClN_3O_4S_2$.

Q. Not less than 80 per cent of the stated amounts of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ and not less than 60 per cent of the stated amounts of $C_7H_8ClN_3O_4S_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 136 mg of *monobasic potassium phosphate* in 800 ml *water*, adjusted to pH 2.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Enalapril Maleate to a 100.0 ml volumetric flask. Add 25.0 ml of buffer solution, and sonicate for 15 minutes. Add 25.0 ml of *methanol*, sonicate for an additional 15 minutes, dilute with buffer solution to volume, and filter.

Reference solution (a). A 0.04 per cent w/v solution of *enalaprilat IPRS* in *water*.

Reference solution (b). Transfer 20 mg of *enalapril maleate IPRS* in a 100-ml beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate at about one-half the maximum temperature setting of hot plate. Heat for about 5 to 10 minutes until the solid is melted. Immediately remove the beaker from the hot plate, and allow cooling. To the cooled residue in the beaker add 50.0 ml of *acetonitrile*, and sonicate for a few minutes to dissolve. The solution typically contains between 0.2 and 0.4 mg per ml of enalapril diketopiperazine.

NOTE — Avoid overheating to prevent heat-induced degradation, which gives rise to a brown colour.

Reference solution (c). Transfer 20 mg of *enalapril maleate IPRS* to a 100.0 ml volumetric flask and dissolve with 25.0 ml of *methanol*. Pipettte 1.0 ml each of reference solution (a) and reference solution (b) into the volumetric flask and dilute with buffer solution to volume.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),

- column temperature: 65°,
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of buffer solution,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

The relative retention times for enalaprilat, enalapril diketopiperazine and enalapril are 0.3, 0.4 and 1.0 respectively.

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 700 theoretical plates for enalapril, 1500 for enalaprilat and 1500 for enalapril diketopiperazine. The tailing factor is not more than 3.5, the resolution between the peaks due to enalapril and any peak is not less than 1.3. The relative standard deviation for replicate injections is not more than 5.0 per cent for enalaprilat and enalapril diketopiperazine and not more than 2.0 per cent for enalapril.

Inject the test solution, the sum of the areas of all impurities is not more than 5.0 per cent, calculated by area normalization.

Uniformity of content. Complies with the test stated under Tablets.

For Enalapril maleate—Determine by liquid chromatography (2.4.14), using the chromatographic system as described under the dissolution of enalapril maleate.

Buffer solution. A solution prepared by dissolving 136 g of *monobasic potassium phosphate* in 800 ml of *water*, adjusted to pH 4.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Solvent mixture. 2 volumes of buffer solution and 98 volumes of *water*.

Test solution. Disperse 1 tablet in solvent mixture with the aid of ultrasound and dilute suitably to obtain a concentration of 0.01 per cent w/v of enalapril maleate. Filter, rejecting the first few ml of the filtrate.

Reference solution. A 0.01 per cent w/v solution of *enalapril maleate IPRS* in the solvent mixture.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the capacity factor is not less than 2.5. The tailing factor is not more than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. *For enalapril maleate* — Determine by liquid chromatography (2.4.14), as described under Related substances test with the following modifications.

Reference solution. Dissolve 20 mg of *enalapril maleate IPRS* in about 25.0 ml of *methanol* and dilute to 100.0 ml with buffer solution.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 700 theoretical plates the tailing factor is not more than 3.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{28}N_2O_5,C_4H_4O_4$ in tablets.

For hydrochlorothiazide — Determine by liquid chromatography (2.4.14),

Buffer solution. A solution prepared by dissolving 136 mg of *monobasic potassium phosphate* in 800 ml *water*, adjusted to pH 2.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Hydrochlorothiazide to a 200-ml volumetric flask. Add 50.0 ml of buffer solution and sonicate for 15 minutes. Add 50.0 ml of *methanol*, sonicate for an additional 15 minutes, dilute with buffer solution to volume, and filter.

Reference solution. A solution prepared by dissolving 10 mg of *hydrochlorothiazide IPRS* in 25.0 ml of *methanol* and dilute to 100.0 ml with buffer solution.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octaylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of buffer solution,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 310 nm,
- injection volume: 50 µl.

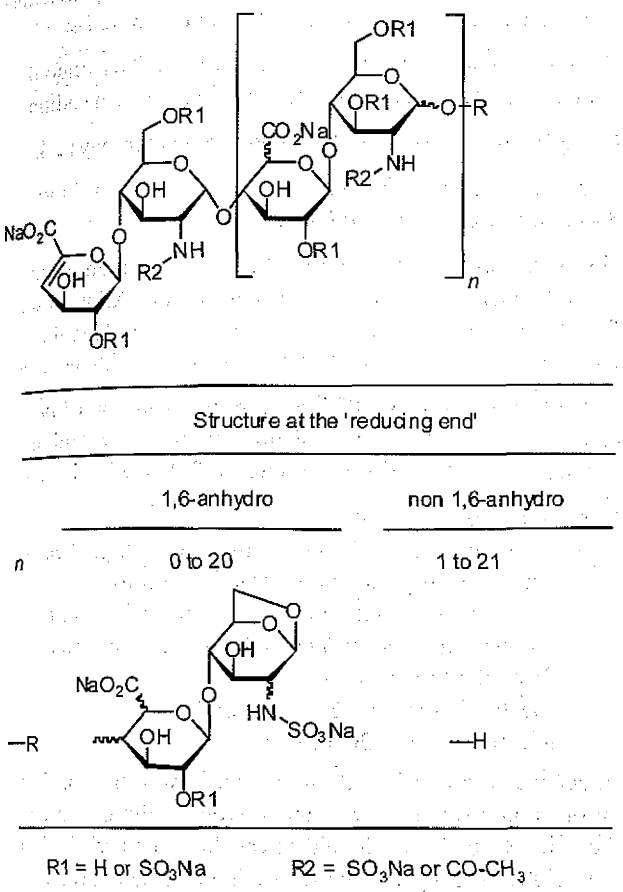
Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the capacity factor is not less than 2.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_7H_8ClN_3O_4S_2$ in tablets.

Storage. Store protected from moisture.

Enoxaparin Sodium



Enoxaparin Sodium is the sodium salt of a low-molecular-mass heparin that is obtained by alkaline depolymerisation of the benzyl ester derivative of heparin from porcine intestinal mucosa. Heparin Sodium used for the manufacture of Enoxaparin complexes with the tests under Heparin Sodium. Enoxaparin consists of a complex set of oligosaccharides that have not yet been completely characterised. Based on current knowledge, the majority of the components have a 4-enopyranose uronate structure at the non- reducing end of their chain. 15 per cent to 25 per cent of the components have a 1, 6- anhydro structure at the reducing end of their chain.

The mass-average relative molecular mass ranges is not less than 3800 and not more than 5000, with a characteristic value of about 4500.

The degree of sulphation is about 2 per disaccharide unit.

The potency is not less than 90 IU and not more than 125 IU of anti-factor X_a activity per milligram, calculated with reference to the dried substance. The anti-factor IIa activity is not less than 20.0 IU and not more than 35.0 IU per milligram, calculated with reference to the dried substance. The ratio of anti-factor X_a activity to anti-factor IIa activity is between 3.3 and 5.3.

The mass-average relative molecular mass ranges is not less than 3800 and not more than 5000. The mass percentage of chains lower than 2000 ranges is not less than 12.0 per cent and not more than 20.0 per cent. The mass percentage of chains is not less than 2000 and not more 8000 ranges is not less than 68.0 per cent and not more than 82.0 per cent.

Category. Anticogulant.

Identification

A. Determine by size-exclusion chromatography (2.4.16).

Test solution. Dissolve 100 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution. A 1.0 per cent w/v solution of *enoxaparin sodium IPRS* in the mobile phase.

Chromatographic system

- two stainless steel column 30 cm x 7.8 mm, packed porous silica beads (5 µm) or hybrid packed with a hydrophilic coating in series with a fractionation range for proteins of approximately 5000 to 150000 Da (Such as TSK gel G20000SW_{XL}), and a guard column 4 cm x 6.0 mm, packed with the same column material (7 µm) (Such as TSK gel SW_{XL}),
- mobile phase. *0.5 M lithium nitrate solution*,
- flow rate: 0.6 ml per minute,
- refractive index detector,
- injection volume: 20 µl.

Reconstitute 1 vial each of enoxaparin sodium molecular weight *calibrant A IPRS* and enoxaparin sodium molecular weight *calibrant B IPRS* in 1 ml of mobile phase. Inject enoxaparin sodium molecular weight *calibrant A IPRS*, enoxaparin sodium molecular weight *calibrant B IPRS* and measure the retention times. Inject in duplicate the reference solution, test solution and record the chromatograms for a length of time to ensure complete elution, including salt and solvent peaks. Calculate the total peak areas under each of the reference solution and test solution chromatograms, excluding salt and solvent peaks at the end.

Plot the retention times on the *x*-axis against the peak molecular weights on the *y*-axis for the peaks from enoxaparin sodium molecular weight calibrant A IPRS and enoxaparin sodium molecular weight calibrant B IPRS, and fit the data to a third-order polynomial, using suitable gel permeation chromatography (GPC) software.

Calculate the data, using the same GPC software; determine the weight-average molecular weight, M_w, for each of the duplicate chromatograms of the reference solution, test solution; and take the average for each solution. Correct the mean value of M_w to the nearest 50. The chromatographic system is suitable if M_w for enoxaparin sodium IPRS is within 150 Da of the labeled M_w value. The M_w for the test solution is between 3800 and 5000 Da. Using the same software, determine for each of the duplicate test solution chromatograms the percentage of enoxaparin sodium chains with molecular weights lower than 2000 Da, M_{2000}, the percentage of enoxaparin sodium chains with molecular weights in the range 2000–8000 Da, $M_{2000-8000}$, and the percentage of enoxaparin sodium chains with molecular weights more than 8000 Da, M_{8000}. Average the duplicate values, and express to the nearest 0.5 per cent.

M_{2000} is between 12.0 per cent and 20.0 per cent, $M_{2000-8000}$ is between 68.0 per cent and 82.0 per cent, and M_{8000} is not more than 18.0 per cent.

B. The ratio of anti-factor Xa activity to anti-factor IIa activity, determined as described under Assay, is between 3.3 and 5.3.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *water* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 6.2 to 7.7, determined in a 10 per cent w/v solution in *carbon dioxide-free water.*

Light absorbance. A 0.05 per cent w/v solution in *0.01 M hydrochloric acid*, determined at 231 nm (2.4.7), shows specific absorbance from 14.0 to 20.0.

Heavy metals (2.3.13). 0.67 g complies with the limit test for heavy metals, Method B (30 ppm).

Sodium. 11.3 per cent to 13.5 per cent.

Determine by atomic absorption spectrometry (2.4.2, Method A).

Test solution. Dissolve 50 mg in *0.1 M hydrochloric acid* containing 0.127 per cent w/v solution of *caesium chloride* and dilute to 100.0 ml with the same solvent.

Reference solution. Prepare reference solutions (25 ppm, 50 ppm and 75 ppm) using *sodium standard solution (200 ppm)* diluted with *0.1 M hydrochloric acid* containing 0.127 per cent w/v solution of *caesium chloride.*

Source. Sodium hollow-cathode lamp.

Wavelength. 330.3 nm.

Atomisation device. Flame of suitable composition (for example, 11 litres of air and 2 litres of acetylene per minute).

Nitrogen (2.3.30). 1.8 to 2.5 per cent, Method E, calculated on dried basis.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven over phosphorous pentaoxide at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Bacterial endotoxins (2.2.3). Not more than 0.01 Endotoxin Unit per IU of anti-factor X_a activity of enoxaparin sodium.

Benzyl alcohol. Determine by liquid chromatography (2.4.14).

Internal standard solution. A 0.1 per cent w/v solution of *3,4-dimethylphenol* in *methanol.*

Test solution. Dissolve about 0.5 g of substance under examination in 5.0 ml of *1 M sodium hydroxide.* Allow to stand for 1 hour. Add 1.0 ml of *glacial acetic acid* and 1.0 ml of the internal standard solution and dilute to 10.0 ml with the *water.*

Reference solution. A 0.025 per cent w/v solution of *benzyl alcohol* in *water.* Mix 0.5 ml of the solution with 1.0 ml of the internal standard solution and dilute to 10.0 ml with the *water.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase. a mixture of 5 volumes of *methanol*, 15 volumes of *acetonitrile* and 80 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 256 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the reference solution, calculate the ratio (R_1) of the height of the peak due to benzyl alcohol to the height of the peak due to the internal standard. In the chromatogram obtained with the test solution, calculate the ratio (R_2) of the height of the peak due to benzyl alcohol to the height of the peak due to the internal standard.

Calculate the percentage content (*m/m*) of benzyl alcohol using the following expression:

$$\frac{0.0125 \times R_2}{m \times R_1}$$

Where, m = mass of the substance under examination, in grams.

The content is not more than 0.1 per cent of benzyl alcohol

Assay (anti-factor X_a activity). Not less than 90 and not more than 125 Anti-Factor X_a IU per mg.

Acetic acid solution. Transfer 42 ml of *glacial acetic acid* to a 100-ml volumetric flask, dilute with *water* to volume, and mix.

Polyethylene glycol 6000 buffer pH 7.4. Dissolve 6.08 g of *tris(hydroxymethyl)aminomethane* and 8.77 g of *sodium*

chloride in 500 ml of *water*. Add 1.0 g of *polyethylene glycol 6000*, adjusted to pH 7.4 with *hydrochloric acid* and dilute with *water* to 1000 ml.

Buffer pH 7.4. Dissolve 6.08 g of *tris(hydroxymethyl) aminomethane* and 8.77 g of *sodium chloride* in 500 ml of *water*. Adjusted to pH 7.4 with *hydrochloric acid* and dilute to 1000 ml with *water*.

Buffer pH 8.4. Dissolve 3.03 g of *tris(hydroxymethyl) aminomethane*, 5.12 g of *sodium chloride* and 1.40 g of *edetate sodium* in 250 ml of *water*. Adjusted to pH 8.4 with *hydrochloric acid* and dilute to 500 ml with *water*.

Human antithrombin III solution. Reconstitute a vial of antithrombin III in *water* to obtain a solution containing 5 Antithrombin III Units per ml. Dilute the solution with *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution having a concentration of 1.0 Antithrombin III Unit per ml.

Factor X_a solution. Reconstitute an weighed quantity of bovine factor X_a in *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution that gives an increase in absorbance value at 405 nm of not more than 0.20 absorbance units per minute when assayed as described below but using as an appropriate volume (V, in µl) of Buffer pH 7.4 instead of V µl of the enoxaparin solution.

Chromogenic substrate solution. Prepare a solution of a suitable chromogenic substrate for amidolytic test for factor X_a in *water* to obtain a concentration of about 3 mM. Dilute with buffer pH 8.4 to obtain a solution having a concentration of 0.5 mM.

Reference solutions. Dilute *Enoxaparin Sodium Solution for Bioassays IPRS* with Buffer pH 7.4 to obtain four dilutions in the concentration range between 0.025 and 0.2 USP Anti-Factor X_a IU per ml.

Test solutions. Proceed as directed for reference solutions to obtain concentrations of Enoxaparin Sodium similar to those obtained for the reference solutions.

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the test solutions; and S1, S2, S3, and S4 each in duplicate for the dilutions of the reference solutions. [*NOTE*—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, V, (20 to 50 µl) of *Human antithrombin III solution* and an equal volume, V, of either the blank, buffer pH 7.4, or an appropriate dilution of the test solutions and reference solutions. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 minute. Add to each tube volume $2V$ (40 to 100 µl) of *Factor* X_a solution, and incubate for 1.0 minute. Add $5V$ (100 to 250 µl) volume of *chromogenic substrate solution*. Stop the reaction after 4.0 minutes with $5V$ (100 to 250 µl) volume of *acetic acid solution*. Measure the absorbance of each solution at 405 nm against blank B1.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and reference solutions, and calculate the potency of the enoxaparin sodium in IU of anti-factor X_a activity per mL using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the anti-factor X_a activity of Enoxaparin Sodium per mg, calculated on the dried basis.

Anti-factor II_a activity. Not less than 20.0 and not more than 35.0 anti-Factor II_a IU per mg.

Acetic acid solution, Polyethylene glycol 6000 buffer pH 7.4, Buffer pH 7.4, Buffer pH 8.4, and *Human antithrombin III solution.* Proceed as directed under *Assay (anti-factor X_a activity)*, except that the concentration of the *Human antithrombin III solution* is 0.5 Antithrombin III Unit per ml.

Thrombin human solution. Reconstitute thrombin human in *water*, and dilute in Polyethylene glycol 6000 buffer pH 7.4 to obtain a solution having a concentration of 5 Thrombin Units per ml.

Chromogenic substrate solution. Prepare a solution of a suitable chromogenic substrate for an amidolytic test for thrombin in *water* to obtain a concentration of about 3 mM. Immediately before use, dilute with buffer pH 8.4 to 0.5 mM.

Reference solutions. Dilute *Enoxaparin Sodium Solution for Bioassays IPRS* with buffer pH 7.4 to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of anti-factor II_a activity per ml.

Test solutions. Proceed as directed under Reference solutions to obtain concentrations of Enoxaparin Sodium similar to those obtained for the Reference solutions.

Proceed as directed under Assay (anti-factor X_a activity), except to use *Thrombin human solution* instead of *Factor X_a solution* and to use the *Human antithrombin III solution* as described above.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and of the reference solutions, and calculate the potency of the enoxaparin sodium in IU of anti-factor II_a activity per mg using statistical methods for parallel-line assays. The four independent dilution estimates are then combined to obtain the final weighted mean. Then calculate the confidence limits. Express the anti-factor II_a activity of Enoxaparin Sodium per mg, calculated on the dried basis.

Anti-factor X_a to anti-factor II_a ratio. The ratio of the numerical value of the anti-factor X_a activity in Anti-Factor X_a IU per mg to the numerical value of the anti-factor II_a activity in Anti-Factor II_a IU per mg, as determined by the Assay (anti-factor

X_a activity) and the Anti-factor II_a activity, respectively, is not less than 3.3 and not more than 5.3.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states (a) the number of International Units of anti-factor X_a activity per miligram; (b) the number of International Units of anti-factor IIa activity per miligram; (c) the mass-average molecular mass and the percentage of molecules within defined molecular mass ranges; (d) where applicable, that the contents are the sodium salt.

Enoxaparin Injection

Enoxaparin Sodium Injection

Enoxaparin Injection is a sterile solution of Enoxaparin Sodium in Water for Injections.

The potency is not less than 90.0 per cent and not more than 110.0 per cent stated in terms of International Anti-factor X_a Units (IU). It may contain, in multiple-dose containers, a suitable antimicrobial preservative, such as benzyl alcohol.

Usual strengths. 20 mg (2000 Anti-factor X_a units); 40 mg (4000 Anti-factor X_a units); 60 mg (6000 Anti-factor X_a units); 80 mg (8000 Anti-factor X_a units).

Identification

A. Add 2 ml of *water* to the total content of a single-dose container or to 0.4 ml from a multiple-dose container, and 1 ml of 2 per cent w/v solution of *protamine sulphate* in a glass test tube, and mix. A creamy white precipitate is formed.

B. Transfer the total content of a single-dose container, or 0.4 ml from a multiple-dose container, dilute to 100 ml with *0.01 M hydrochloric acid* and 0.05 per cent w/v of reference solution shows absorption maxima at 231 nm (2.4.7).

C. Complies with the test for sodium (2.3.1).

Tests

pH (2.4.24). 5.5 to 7.5.

Benzyl alcohol *(if present)*. 1.35 per cent to 1.65 per cent.

Test solution. Dilute 5.0 ml of the Injection to 50 ml with the mobile phase.

Reference solution. Dissolve about 75 mg of *benzyl alcohol IPRS* in 50 ml of the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *water*, 15 volumes of *acetonitrile* and 5 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 256 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of benzyl alcohol.

Free sulphate. Not more than 0.12 per cent.

Determine by ion chromatography (2.4.14).

Test solution. Transfer about 200 mg of a 100 mg per ml Injection, weighed, to a suitable previously tared sulfate-free vial. Add mobile phase to obtain a total mass of about 20 g.

Sulphate stock solution. Dissolve an accurately weighed quantity of *sodium sulphate* equivalent to 0.1 g of *sulphate* in 100.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 25.0 ml with the mobile phase.

Reference solution. Prepare solutions of 0.1 µg per ml, 0.5 µg per ml, 1 µg per ml, 2 µg per ml, 4 µg per ml, and 5 µg per ml by appropriate dilution of the sulphate stock solution in the mobile phase.

System suitability solution. Prepare a solution containing 3 µg per ml of sulphate anion and 5 µg per ml of oxalate anion.

Chromatographic system
- a column 25 cm x 4 mm, packed with anion-exchange resin and a 5 cm x 4 mm anion-exchange guard column, both containing ethylvinylbenzene cross linked with 55 per cent divinylbenzene with latex coating of microbeads bonded with alkanol quaternary ammonium ions (6 per cent),
- mobile phase: a *3.0 mM sodium carbonate solution*,
- flow rate: 2.0 ml per minute,
- conductivity detector,
- injection volume: 25 µl.

Inject system suitability solution. The test is not valid unless the resolution between the peak due to sulphate and oxalate is not less than 1.

Inject the reference solution and the test solution.

Other tests. Comply with the tests stated under Parenteral Peparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 0.01 Endotoxin Unit per unit of anti-factor X_a activity in Anti-factor X_a IU.

Assay (anti-factor X_a activity). The estimated activity is not less than 90.0 per cent and not more than 110.0 per cent of the stated activity.

Acetic acid solution. Transfer 42 ml of *glacial acetic acid* to a 100-ml volumetric flask, dilute with *water* to volume, and mix.

Polyethylene glycol 6000 buffer pH 7.4. Dissolve 6.08 g of *tris(hydroxymethyl)aminomethane* and 8.77 g of *sodium*

chloride in 500 ml of *water*. Add 1.0 g of *polyethylene glycol 6000*, adjusted to pH 7.4 with *hydrochloric acid* and dilute with *water* to 1000.0 ml.

Buffer pH 7.4. Dissolve 6.08 g of *tris(hydroxymethyl) aminomethane* and 8.77 g of *sodium chloride* in 500 ml of *water*. Adjusted to pH 7.4 with *hydrochloric acid* and dilute to 1000.0 ml with *water*.

Buffer pH 8.4. Dissolve 3.03 g of *tris(hydroxymethyl) aminomethane*, 5.12 g of *sodium chloride* and 1.40 g of *edetate sodium* in 250 ml of *water*. Adjusted to pH 8.4 with *hydrochloric acid* and dilute to 500.0 ml with *water*.

Human antithrombin III solution. Reconstitute a vial of antithrombin III in *water* to obtain a solution containing 5 Antithrombin III Units per ml. Dilute the solution with *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution having a concentration of 1.0 Antithrombin III Unit per ml.

Factor X_a solution. Reconstitute an weighed quantity of bovine factor X_a in *Polyethylene glycol 6000* buffer pH 7.4 to obtain a solution that gives an increase in absorbance value at 405 nm of not more than 0.20 absorbance units per minute when assayed as described below but using as an appropriate volume (V, in µl) of Buffer pH 7.4 instead of V µl of the enoxaparin solution.

Chromogenic substrate solution. Prepare a solution of a suitable chromogenic substrate for amidolytic test for factor X_a in *water* to obtain a concentration of about 3 mM. Dilute with buffer pH 8.4 to obtain a solution having a concentration of 0.5 mM.

Reference solutions. Dilute *Enoxaparin Sodium Solution for Bioassays IPRS* with Buffer pH 7.4 to obtain four dilutions in the concentration range between 0.025 and 0.2 USP Anti-Factor X_a IU per ml.

Test solutions. Proceed as directed for reference solutions to obtain concentrations of Enoxaparin Sodium similar to those obtained for the reference solutions.

Label 18 suitable tubes: B1 and B2 for blanks; T1, T2, T3, and T4 each in duplicate for the dilutions of the test solutions; and S1, S2, S3, and S4 each in duplicate for the dilutions of the reference solutions. [*NOTE*—Treat the tubes in the order B1, S1, S2, S3, S4, T1, T2, T3, T4, T1, T2, T3, T4, S1, S2, S3, S4, B2.] To each tube add the same volume, V, (20 to 50 µl) of *Human antithrombin III solution* and an equal volume, V, of either the blank, buffer pH 7.4, or an appropriate dilution of the test solutions and reference solutions. Mix, but do not allow bubbles to form. Incubate at 37° for 1.0 minute. Add to each tube volume $2V$ (40 to 100 µl) of *Factor* X_a solution, and incubate for 1.0 minute. Add $5V$ (100 to 250 µl) volume of *chromogenic substrate solution*. Stop the reaction after 4.0 minutes with $5V$ (100 to 250 µl) volume of *acetic acid solution*. Measure the absorbance of each solution at 405 nm against blank B1.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and reference solutions, and calculate the potency of the enoxaparin sodium in IU of anti-factor X_a activity per mL using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the anti-factor X_a activity of Enoxaparin Sodium per ml.

Anti-factor II_a activity. Not less than 2000 and not more than 3500 anti-Factor II_a IU per ml.

Acetic acid solution, Polyethylene glycol 6000 buffer pH 7.4, Buffer pH 7.4, Buffer pH 8.4, and *Human antithrombin III solution.* Proceed as directed under *Assay (anti-factor X_a activity),* except that the concentration of the *Human antithrombin III solution* is 0.5 Antithrombin III Unit per ml.

Thrombin human solution. Reconstitute thrombin human in *water*, and dilute in Polyethylene glycol 6000 buffer pH 7.4 to obtain a solution having a concentration of 5 Thrombin Units per ml.

Chromogenic substrate solution. Prepare a solution of a suitable chromogenic substrate for an amidolytic test for thrombin in *water* to obtain a concentration of about 3 mM. Immediately before use, dilute with buffer pH 8.4 to 0.5 mM.

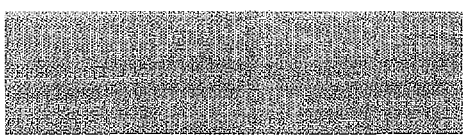
Reference solutions. Dilute *Enoxaparin Sodium Solution for Bioassays IPRS* with buffer pH 7.4 to obtain four dilutions having concentrations in the range between 0.015 and 0.075 IU of anti-factor II_a activity per ml.

Test solutions. Proceed as directed under Reference solutions to obtain concentrations of Enoxaparin Sodium similar to those obtained for the Reference solutions.

Proceed as directed under Assay (anti-factor X_a activity), except to use *Thrombin human solution* instead of *Factor X_a solution* and to use the *Human antithrombin III solution* as described above.

For each series, calculate the regression of the absorbance against log concentrations of the test solutions and of the reference solutions, and calculate the potency of the enoxaparin sodium in IU of anti-factor II_a activity per ml using statistical methods for parallel-line assays. The four independent dilution estimates are then combined to obtain the final weighted mean. Then calculate the confidence limits. Express the anti-factor II_a activity of Enoxaparin Sodium per ml.

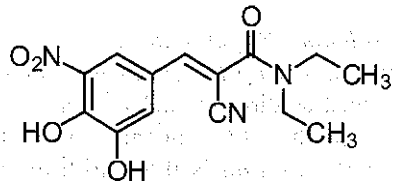
Anti-factor X_a to anti-factor II_a ratio. The ratio of the numerical value of the anti-factor X_a activity in Anti-Factor X_a IU per ml to the numerical value of the anti-factor II_a activity in Anti-Factor II_a IU per ml, as determined by the Assay (anti-factor



X_a activity) and the Anti-factor II_a activity, respectively, is not less than 3.3 and not more than 5.3.

Labelling. It indicates the amount (mg) of Enoxaparin Sodium in the total volume of contents. The label states also that the Enoxaparin Sodium starting material is porcine derived.

Entacapone



$C_{14}H_{15}N_3O_5$ Mol.Wt. 305.3

Entacapone is (2*E*)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N,N*-diethyl-2-propenamide.

Entacapone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{15}N_3O_5$,calculated on the dried basis.

Category. Antiparkinson.

Identification

A. Determine by infrared absorption spectrophotometery (2.4.6).Compare the spectrum with that obtained with *entacapone IPRS* or with the reference spectrum of entacapone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *methanol* and 30 volumes of *tetrahydrofuran*.

Test solution. Dissolve 0.1g of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A solution containing 0.0001 per cent w/v each of *entacapone impurity A IPRS ((Z)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethylacrylamide IPRS)* and *entacapone IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl bonded to porous silica (5 µm),
- mobile phase: a mixture of 22 volumes of *methanol*, 1 volume of *tetrahydrofuran* and 27 volumes of a 0.234 per cent w/v solution of *monobasic sodium phosphate dihydrate* adjusted to pH 2.1 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 10 µl.

The relative retation time with reference to entacapone for entacapone impurity A is 0.8.

Inject the reference solution. The test is not valid unless the resolution between the peaks corresponding to entacapone impurity A and entacapone is not less than 2.0.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to entacapone impurity A is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks other than entacapone impurity A is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 65° at a pressure not exceeding 49 mm of Hg.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *methanol* and 30 volumes of *tetrahydrofuran*.

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.0001 per cent w/v each of *entacapone impurity A IPRS* and *entacapone IPRS* with the solvent mixture.

Reference solution (b). A 0.01 per cent w/v solution of *entacapone IPRS* in the solvent mixture.

Use the chromatographic system as described under Related substances.

The relative retation time with reference to entacopane for entacapone impurity A is about 0.8.

Inject reference solution (a). The test is not valid unless the resolution between the peaks corresponding to entacapone impurity A and entacapone is not less than 2.0.

Inject reference solution (b). The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent for entacapone peak.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{14}H_{15}N_3O_5$.

Storage. Store protected from moisture.

Entacapone Tablets

Entacapone Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of entacapone, $C_{14}H_{15}N_3O_5$.

Usual strength. 200 mg.

Identification

A. Disperse a quantity of powdered tablets containing 0.05 g of entacapone with 50 ml of *methanol*, sonicate for about 30 minutes and filter. Evaporate the filterate to dryness on a water-bath and dry the residue in an oven at 105° for about half an hour. The residue complies with the following test

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *entacapone IPRS* or with the reference spectrum of entacapone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of phosphate buffer, pH 5.5 prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 5.5 with *1 M sodium hydroxide*,

Speed and time. 50 rpm and 30 minutes.

NOTE — Protect the solutions from light.

Reference solution. A 0.022 per cent w/v solution of *entacapone IPRS*, prepared by dissolving in *tetrahydrofuran* upto 2 per cent of the volume and diluting with dissolution medium to volume. Dilute 5.0 ml of the solution to 50.0 ml with the dissolution medium.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with medium, if necessary, at the maximum at about 313 nm (2.4.7). Calculate the content of $C_{14}H_{15}N_3O_5$ in the medium from the absorbance obtained from the reference solution using a mixture of 0.2 volume of *tetrahydrofuran* and 99.8 volumes of medium as blank.

Q. Not less than 80 per cent of the stated amount of $C_{14}H_{15}N_3O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Solvent mixture. 70 volumes of *methanol* and 30 volumes of *tetrahydrofuran*.

Test solution. Disperse a quantity of powdered tablets containing 0.3 g of Entacapone with 30 ml of *tetrahydrofuran* with the aid of ultrasound and dilute to 100.0 ml with *methanol*, centrifuge. *(NOTE — Prepare the test solution fresh and use within 7 hours of preparation).*

Reference solution (a). A solution containing 0.003 per cent w/v each of *entacapone IPRS* and *entacapone impurity A IPRS* in the solvent mixture.

Reference solution (b). A 0.0003 per cent w/v solution of *entacapone IPRS* in the solvent mixture.

Use chromatographic system as described under Assay.

Name	Relative retention time
Entacapone impurity A[1]	0.8
Entacapone	1.0

[1](*Z*)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N*,*N*-diethylacrylamide.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to entacapone impurity A and entacapone is not less than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to entacapone impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks other than entacapone impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Solvent mixture. 70 volumes of *methanol* and 30 volumes of *tetrahydrofuran*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of Entacapone in 30 ml of *tetrahydrofuran* with the aid of ultrasound and dilute to 100.0 ml with *methanol*, centrifuge.

Reference solution. A 0.05 per cent w/v solution of *entacapone IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 22 volumes of *methanol*, 1 volume of *tetrahydrofuran* and 27 volumes of buffer solution prepared by dissolving 2.1 g of *monobasic sodium phosphate* in 1000 ml of *water*, adjusted to pH 2.1 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

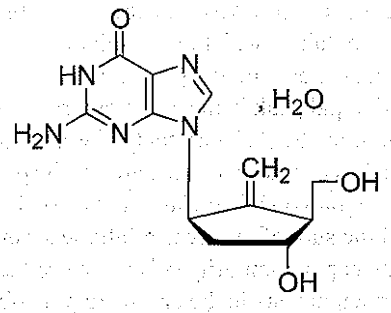
Inject the reference solution and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak.

Calculate the content of $C_{14}H_{15}N_3O_5$ in the tablets.

Storage. Store protected from light at a temperature not exceeding 30°.

Entecavir

Entecavir Monohydrate



$C_{12}H_{15}N_5O_3,H_2O$ Mol.Wt.295.3

Entecavir is 2-amino-1,9-dihydro-9-[(1*S*,3*R*,4*S*)-4hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl]-6*H*-purin-6-one,monohydrate.

Entecavir contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{15}N_5O_3$, calculated on the anhydrous basis.

Category. Antiviral.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *entecavir monohydrate IPRS* or with the reference spectrum of entecavir monohydrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +25.0° to +30.0°, determined in a 1.0 per cent w/v solution in a mixture of 1 volume of *dimethyl formamide* and 1 volume of *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of mobile phase A and 10 volumes of mobile phase B.

Test solution. Dissolve 25 mg of the substance under examination in 2.5 ml of *methanol* and dilute to 25.0 ml with mobile phase A.

Reference solution. Dissolve 25 mg of *entecavir monohydrate IPRS* in 2.5 ml of *methanol* and dilute to 25.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture. Further dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *0.01M sodium dihydrogen phosphate*, adjusted to pH 3.0 with *orthophosphoric acid*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
5	95	5
30	30	70
60	30	70
60.1	95	5
80	95	5

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent); the sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 5.5 to7.0 per cent, determined on 0.1g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of buffer solution and 10 volumes of *methanol.*

Test solution. Dissolve 50 mg of the substance under examination in 5 ml of *methanol* and dilute to 50.0 ml with the buffer solution. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. Dissolve 50 mg of *entecavir monohydrate IPRS* in 5 ml of *methanol* and dilute to 50.0 ml with the buffer solution. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of buffer solution of *0.01M sodium dihydrogen phosphate*, adjusted to pH 3.0 with *orthophosphoric acid* and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{12}H_{15}N_5O_3$.

Storage. Store at a temperature not exceeding 30°.

Entecavir Tablets

Entecavir Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of entecavir, $C_{12}H_{15}N_5O_3$.

Usual strengths. 0.5 mg; 1.0 mg.

Identification

In the Assay, the retention time of principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium.1000 ml of *phosphate buffer pH 6.8,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *entecavir monohydrate IPRS* in the mobile phase and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *water* and 20 volumes of *methanol,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

Inject the reference solution and the test solution.

Q. Not less than 75 per cent of the stated amount of $C_{12}H_{15}N_5O_3$.

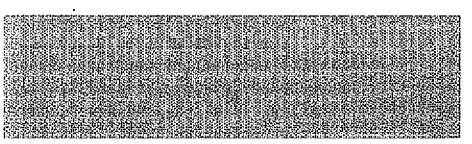
Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of *water* and 10 volumes of *methanol.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 10 mg of entecavir in the solvent mixture, mix with the aid of ultrasound for 30 minutes and dilute to 100.0 ml with the solvent mixture. Centrifuge this solution at 3000 rpm for 10 minutes and filter.

Reference solution. A 0.0001 per cent w/v solution of *entecavir monohydrate IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,



- mobile phase: A. dissolve 0.77 g of *ammonium acetate* and 1 ml of *hexylamine* in 1000 ml *water*, adjusted to pH 6.5 with *glacial acetic acid*,
  B. a mixture of 75 volumes of *methanol* and 25 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
6	95	5
15	85	15
23	85	15
24	95	5
30	95	5

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution. (0.1 per cent).

Uniformity of content. Complies with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using following modification.

Test solution. Disperse one tablet in 6 ml of the mobile phase. Mix with the aid of ultrasound for 15 minutes. Dilute to 10 ml with mobile phase. Dilute as necessary to obtain a solution containing 0.005 per cent w/v of entecavir.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 5 mg of entecavir in the mobile phase with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the mobile phase and filter.

Reference solution. A 0.005 per cent w/v solution of *entecavir monohydrate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil C18),
- mobile phase: a mixture of 80 volumes of *water* and 20 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

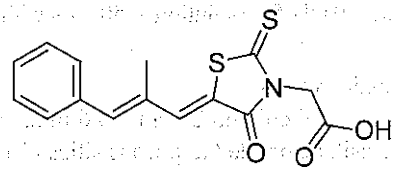
Inject the reference solution and the test solution.

Calculate the content of $C_{12}H_{15}N_5O_3$ in the tablets.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label state the strength in terms of equivalent amount of entecavir.

Epalrestat



$C_{15}H_{13}NO_3S_2$ Mol. Wt. 319.4

Epalrestat is 2-{(5Z)-5-[(2E)-2-Methyl-3-phenylprop-2-en-1-ylidene]-4-oxo-2-thioxothiazolidin-3-yl}acetic acid.

Epalrestat contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{15}H_{13}NO_3S_2$, calculated on the dried basis.

Category. Antidiabetic.

Description. A yellow to orange crystalline powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *epalrestat IPRS* or with the reference spectrum of epalrestat.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.0005 per cent w/v solution in *methanol* shows absorption maxima and minima at the same wavelength as that of *epalrestat IPRS* prepared in the same manner.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the tests protected from light.

Test solution. Dissolve 25 mg of the substance under examination in 10.0 ml of *N,N-dimethylformamide.*

Reference solution. A 0.0025 per cent w/v solution of *epalrestat IPRS* in *N,N-dimethylformamide.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* and 7.09 g of *disodium hydrogen phosphate* in 1000 ml of *water*, adjusted to pH 6.5 and 35 volumes of *acetonitrile,*
- flow rate: Adjust, so that the retention time of epalrestat is about 12 minutes,
- spectrophotometer set at 280 nm,
- injection volume: 3 µl.

Inject the reference solution. The test is not valid unless the column efficiency in not less than 6000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times of the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.2 per cent, determined on 1.0 g by drying under vacuum over silica gel at 60° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the tests protected from light.

Internal standard solution. A 1.0 per cent v/v solution of *propyl parahydroxy benzoate* in *N,N-dimethylformamide.*

Test solution. Dissolve 20 mg of the substance under examination in 2.0 ml of the internal standard solution and dilute to 10.0 ml with *N,N-dimethylformamide.* Dilute 1.0 ml of the solution to 10.0 ml with *N,N-dimethylformamide.*

Reference solution. Dissolve 20 mg of *epalrestat IPRS* in 2.0 ml of the internal standard solution and dilute to 10.0 ml with *N,N-dimethylformamide.* Dilute 1.0 ml of the solution to 10.0 ml with *N,N-dimethylformamide.*

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of the ratio of peak area of epalrestat and internal standard for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{13}NO_3S_2$ using the ratio of peak area of epalrestat and internal standard.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Epalrestat Tablets

Epalrestat Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of epalrestat, $C_{15}H_{13}NO_3S_2$.

Usual strength. 50 mg.

Identification

Extract a quantity of the powdered tablets containing 50 mg of Epalrestat, with 100.0 ml of *methanol*, filter. Dilute 1.0 ml of the filtrate to 100.0 ml with *methanol.* When examined in the range 200 nm to 400 nm (2.4.7), the solution exhibits maxima between 235 nm and 239 nm, between 290 nm and 294 nm and between 387 nm and 391 nm.

Tests

NOTE — Carry out the tests protected from light.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of equal volumes of *phosphate buffer pH 6.8* and *water,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute a suitable volume of the filtrate with dissolution medium, measure the absorbance of the resulting solution at the maximum at about 398 nm (2.4.7). Calculate the content of epalrestat, $C_{15}H_{13}NO_3S_2$ in the medium from the absorbance obtained from a solution of known concentration of *epalrestat IPRS* in the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{15}H_{13}NO_3S_2$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. A 1.0 per cent v/v solution of *propyl parahydroxy benzoate* in *N,N-dimethylformamide.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 200 mg of Epalrestat in 20.0 ml of the internal standard solution and dilute to 100.0 ml with *N,N-dimethylformamide.* Dilute 1.0 ml of the solution to 10.0 ml with *N,N-dimethylformamide.*

Reference solution. Dissolve 20 mg of *epalrestat IPRS* in 2.0 ml of the internal standard solution and dilute to 10.0 ml with *N,N-dimethylformamide.* Dilute 1.0 ml of the solution to 10.0 ml with *N,N-dimethylformamide.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of the buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* and 7.09 g of *disodium hydrogen phosphate* in 1000 ml of *water*, adjusted to pH 6.5 and 35 volumes of *acetonitrile,*
- flow rate: adjust, so that the retention time of epalrestat is about 12 minutes,
- spectrophotometer set at 280 nm,
- injection volume: 3 µl.

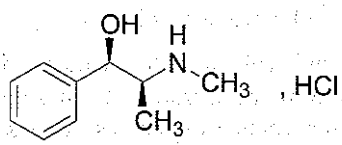
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of the ratio of peak area of epalrestat and internal standard for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{13}NO_3S_2$ in the tablets, using the ratio of peak area of epalrestat and internal standard.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Ephedrine Hydrochloride



$C_{10}H_{15}NO,HCl$ Mol. Wt. 201.7

Ephedrine Hydrochloride is (1*R*,2*S*)-2-methylamino-1-phenylpropan-1-ol hydrochloride.

Ephedrine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{15}NO,HCl$ calculated on the dried basis.

Category. Sympathomimetic; bronchodilator.

Description. Colourless crystals or a white, crystalline powder. It is affected by light.

Identification

Tests A, C and D may be omitted if tests B and E are carried out. Test B may be omitted if tests A, C, D and E are carried out.

Solution A. A 10.0 per cent w/v solution in *water*.

A. Specific optical rotation (see Tests).

B. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ephedrine hydrochloride IPRS* or with the reference spectrum of ephedrine hydrochloride.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Test solution. Dissolve 20 mg of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol.*

Reference solution. A 0.2 per cent w/v solution of *ephedrine hydrochloride IPRS* in *methanol.*

Mobile phase. A mixture of 5 volumes of *dichloromethane,* 15 volumes of *concentrated ammonia* and 80 volumes of *2-propanol.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. After development, dry the plate in air and spray with *ninhydrin solution*, heat at 110° for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. To 0.1 ml of solution A, add 1 ml of *water*, 0.2 ml of *copper sulphate solution* and 1 ml of *strong sodium hydroxide solution*. A violet colour is produced. Add 2 ml of *dichloromethane* and shake. The lower (organic) layer is dark grey and the upper (aqueous) layer is blue.

E. To 5 ml of solution A, add 5 ml of *water*. The solution gives reaction (a) of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution (Solution A) is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of Solution A add 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*; the solution is red.

Specific optical rotation (2.4.22). −35.5° to −33.5°, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 75 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v each of the substance under examination and *pseudoephedrine hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with phenylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 6 volumes of *methanol* and 94 volumes of a 1.16 per cent w/v solution of *ammonium acetate* adjusted to pH 4.0 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 257 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Ephedrine (retention time: about 8 minutes)	1.0	—
Ephedrine impurity B[1]	1.1	—
Ephedrine impurity A[2]	1.4	0.4

[1] pseudoephedrine,

[2] (-)-(1*R*)-1-hydroxy-1-phenylpropan-2-one.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to ephedrine and ephedrine impurity B is not less than 2.0.

Inject reference solution (a) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to ephedrine impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks other than ephedrine impurity A is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphates (2.3.17). 15 ml of solution A complies with the limit test for sulphates (100 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.17 g, dissolve in 10 ml of *mercuric acetate solution*, warming gently, add 50 ml of *acetone* and mix. Titrate with *0.1 M perchloric acid*, using 1 ml of a saturated solution of *methyl orange* in *acetone* as indicator, until a red colour is obtained. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02017 g of $C_{10}H_{15}NO,HCl$.

Storage. Store protected from light.

Ephedrine Nasal Drops

Ephedrine Nasal Drops are a solution of Ephedrine Hydrochloride in a suitable aqueous vehicle.

Ephedrine Nasal Drops contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$.

Usual strengths. 0.5 per cent; 0.75 per cent; 1.0 per cent.

Identification

A. To a quantity of the nasal drops containing 0.1 g of Ephedrine Hydrochloride, add 2 ml of *2 M hydrochloric acid*, shake with two 20 ml quantities of *chloroform* and discard the chloroform. Make the aqueous layer alkaline with *5 M ammonia* and extract with two quantities, each of 30 ml, of a mixture of 3 volumes of *chloroform* and 1 volume of *ethanol (95 per cent)*. Dry the combined extracts over *anhydrous sodium sulphate*, filter and evaporate to dryness at a pressure of 2 kPa, heating gently to remove the last traces of solvent. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ephedrine hydrochloride IPRS*, treated in the same manner or with the reference spectrum of ephedrine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

pH (2.4.24). 4.0 to 7.0.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *2-propanol*, 15 volumes of *13.5 M ammonia solution* and 5 volumes of *chloroform*.

Test solution (a). Dilute the nasal drops, if necessary, with *water* to obtain 0.5 per cent w/v of Ephedrine Hydrochlooride.

Test solution (b). Dilute 1.0 ml of test solution (a) to 5.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of test solution (a) to 200.0 ml with *water*.

Reference solution (b). A 0.1 per cent w/v solution of *ephedrine hydrochloride IPRS* in *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 100° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Other tests. Comply with the tests stated under Nasal Preparations.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the nasal drops with *methanol* to obtain 0.1 per cent w/v solution of Ephedrine Hydrochloride.

Reference solution. A 0.1 per cent w/v solution of *ephedrine hydrochloride IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such Nucleosil C18),
- mobile phase: *0.005 M dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 263 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{15}NO,HCl$ in the nasal drops.

Ephedrine Oral Solution

Ephedrine Hydrochloride Oral Solution; Ephedrine Hydrochloride Elixir; Ephedrine Elixir

Ephedrine Oral Solution is a solution containing 0.3 per cent w/v of Ephedrine Hydrochloride in a suitable flavoured vehicle containing a sufficient volume of Ethanol (95 per cent) or of an appropriate dilute ethanol to give a final concentration of not more than 3 per cent v/v of ethanol.

Ephedrine Oral Solution contains not less than 0.27 per cent and not more than 0.33 per cent w/v of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$.

Identification

A. To 30 ml add 2 ml of *2 M hydrochloric acid*, extract with two quantities, each of 20 ml, of *ether* and discard the ether. Add sufficient *dilute ammonia solution* to the aqueous phase to make it alkaline, extract with two quantities, each of 30 ml, of *ether*, wash the combined ether extracts with three quantities, each of 15 ml, of *water*, dry over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ephedrine hydrochloride IPRS* treated in the same manner or with the reference spectrum of ephedrine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Ethanol content. Not more than 3 per cent v/v, determined by gas chromatography (2.4.13).

Test solution. Use the preparation under examination.

Reference solution (a). Add sufficient of *1-propanol* (internal standard) to the test solution to produce a solution containing 3.0 per cent v/v of *1-propanol*.

Reference solution (b). A 3.0 per cent v/v solution each of internal standard and *ethanol* in *water*.

Chromatographic system
- a column 1.5 m x 4 mm, packed with porous polymer beads (100 to 200 mesh) (Such as Porapak Q and Chromosorb 101),
- temperature:
  column 150°,
  inlet port and detector. 170°,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of reference solution (a), (b) and the test solution.

Calculate the content of ethanol from the areas of the peaks due to ethanol in the chromatograms obtained with reference solution (a) and (b).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *2-propanol*, 15 volumes of *strong ammonia solution* and 5 volumes of *chloroform*.

Test solution (a). Add sufficient *5 M ammonia* to 50 ml of the oral solution to make it alkaline, extract with two quantities, each of 100 ml, of *ether,* wash the combined extracts with 10 ml of *water,* dry with *anhydrous sodium sulphate,* filter and evaporate the filtrate to dryness. Dissolve the oily residue in sufficient *methanol* to produce 5 ml.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol.*

Reference solution (a). Dilute 1.0 ml of test solution (a) to 200.0 ml with *methanol.*

Reference solution (b). A 0.3 per cent w/v solution of *ephedrine hydrochloride IPRS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a weighed quantity of the oral solution containing about 60 mg of Ephedrine Hydrochloride to 50 ml with *methanol.*

Reference solution. A 0.12 per cent w/v solution of *ephedrine hydrochloride IPRS* in *methanol.*

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: *0.005 M dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol,* 35 volumes of *water* and 1 volume of *glacial acetic acid,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 263 nm,
- injection volume: 20 µl.

Determine the weight per ml of the oral solution (2.4.29), and calculate the content of $C_{10}H_{15}NO,HCl$, weight in volume.

Storage. Store protected from light.

Ephedrine Tablets

Ephedrine Hydrochloride Tablets

Ephedrine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ephedrine hydrochloride, $C_{10}H_{15}NO,HCl$.

Usual strengths. 15 mg; 30 mg; 60 mg.

Identification

A. Shake a quantity of the powdered tablets containing about 0.1 g of Ephedrine Hydrochloride with 20 ml of *0.1 M hydrochloric acid,* filter, wash the filtrate with two quantities, each of 20 ml, of *chloroform* and discard the chloroform. Make the aqueous layer alkaline with *5 M ammonia* and extract with two quantities, each of 30 ml, of a mixture of 3 volumes of *chloroform* and 1 volume of *ethanol (95 per cent).* Dry the combined extracts over *anhydrous sodium sulphate,* filter and evaporate to a low volume at a pressure of 2 kPa. Prepare a disc using 0.3 g of *potassium bromide IR,* apply dropwise to the disc 0.1 ml of the *chloroform* solution, allowing the solvent to evaporate between applications, and dry the disc at 50° for 2 minutes. The disc so obtained complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ephedrine hydrochloride IPRS* treated in the same manner or with the reference spectrum of ephedrine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Triturate a quantity of the powdered tablets containing about 0.4 g of Ephedrine Hydrochloride with 10 ml of *chloroform* and discard the *chloroform.* Repeat trituration with a further 10 ml of *chloroform* and again discard the *chloroform.* Shake the residue with 30 ml of warm *ethanol (95 per cent)* for 20 minutes, filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 80° (residue R). Dissolve 10 mg of residue R in 1 ml of *water* and add 0.1 ml of *cupric sulphate solution* followed by 1 ml of *sodium hydroxide solution;* a violet colour is produced. Add 1 ml of *ether* and shake; the ether layer is purple and the aqueous layer is blue.

D. A 5.0 per cent w/v solution of residue R gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 80 volumes of *2-propanol,* 15 volumes of *strong ammonia solution* and 5 volumes of *chloroform.*

Test solution (a). Extract a quantity of the powdered tablets containing 0.1 g of Ephedrine Hydrochloride with 5 ml of *methanol* and filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol.*

Reference solution (a). Dilute 1.0 ml of test solution (a) to 200.0 ml with *methanol.*

Reference solution (b). A 0.2 per cent w/v solution of *ephedrine hydrochloride IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot of lighter colour than the background.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Ephedrine Hydrochloride, shake with 30 ml *methanol* for 10 minutes, add sufficient *water* to produce 50.0 ml, filter through glass fibre (Whatman GF/C is suitable) and use the filtrate.

Reference solution. A 0.1 per cent w/v solution of *ephedrine hydrochloride IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a 0.005 M solution of *dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 263 nm,
- injection volume: 20 µl.

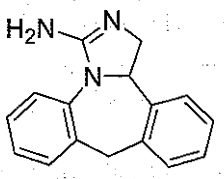
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{15}NO,HCl$ in the tablets.

Storage. Store protected from light.

Epinastine Hydrochloride



$C_{16}H_{15}N_3,HCl$ Mol. Wt. 285.8

Epinastine Hydrochloride is (*RS*)-3-Amino-9,13b-dihydro-1*H*-dibenz[c,f]imidazo[1,5-*a*]azepine hydrochloride.

Epinastine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{15}N_3,HCl$, calculated on the dried basis.

Category. Antihistaminic

Description. A white or almost white, hygroscopic, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *epinastine hydrochloride IPRS* or with the reference spectrum of epinastine hydrochloride.

B. It gives reaction (a) of chlorides (2.3.1).

Tests

Acidity or alkalinity. Dissolve 1.0 g in *carbon dioxide-free water* and dilute to 10 ml with the same solvent. Add 0.1 ml of *methyl red solution* and 0.25 ml of *0.01 M sodium hydroxide*; the solution is green. Add 0.5 ml of *0.01 M hydrochloric acid*; the solution is reddish-violet.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of mobile phase B and 75 volumes of mobile phase A.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. Dilute 10.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 3.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 50°,
- mobile phase: A. a mixture of 15 volumes of *methanol* and 85 volumes of buffer solution prepared by dissolving 3.8 g of *sodium pentanesulphonate monohydrate* and 4.0 g of *potassium dihydrogen phosphate* in *water*, adjusted to pH 4.4 with *orthophosphoric acid* and dilute to 1000 ml with *water*,
  B. a mixture of 15 volumes of *methanol* and 85 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent w/v)
0	80	20
4	80	20
13	30	70
15	80	20
20	80	20

Name	Relative retention time
Epinastine (Retention time: about 4 minutes)	1.0
Epinastine impurity A[1]	1.2
Epinastine impurity B[2]	2.0

[1] 9*H*-dibenzo[*c*,*f*]imidazo[1,5-*a*]azepin-3-amine,

[2] (13b*RS*)-7-bromo-9,13b-dihydro-1*H*-dibenzo[*c*,*f*]imidazo[1,5-*a*]azepin-3-amine.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to epinastine impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent), the area of any peak corresponding to epinastine impurity A is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 7 times the area of the principal peak in the chromatogram obtained with the reference solution (0.7 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.2 g, dissolve in 100 ml of a mixture of 1 volume of *anhydrous acetic acid* and 2 volumes of *acetic anhydride*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02858 g of $C_{16}H_{16}ClN_3$.

Storage. Store protected from moisture.

Epinastine Eye Drops

Epinastine Hydrochloride Eye Drops

Epinastine Eye Drops is a sterile solution of Epinastine Hydrochloride in Purified Water.

Epinastine Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of epinastine hydrochloride, $C_{16}H_{15}N_3$, HCl.

Usual strength. 0.05 per cent w/v.

Identification

In the Assay, the principle peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.5 to 7.5.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal mixture of *water* and *acetonitrile*.

Test solution. Dilute a volume of eye drops to obtain a solution containing 0.05 per cent w/v Epinastine Hydrochloride in the solvent mixture.

Reference solution. A 0.05 per cent w/v solution of *epinastine hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 90 volumes of *water*, 10 volumes of *acetonitrile* and 0.1 volume of *trifluoroacetic acid*,
  B: a mixture of 10 volumes of *water*, 90 volumes of *acetonitrile* and 0.1 volume of *trifluoroacetic acid*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent w/v)
0	85	15
16	75	25
38	25	75
43	25	75
45	85	15
50	85	15

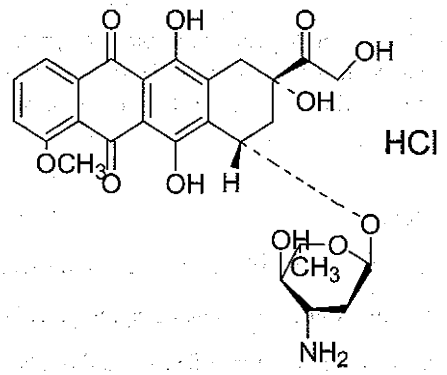
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{15}N_3$, HCl in the eye drops.

Storage. Store protect from light.

Epirubicin Hydrochloride



$C_{27}H_{30}ClNO_{11}$ Mol. Wt. 580.0

Epirubicin Hydrochloride is (8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-*L-arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride.

Epirubicin Hydrochloride contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{27}H_{30}ClNO_{11}$, calculated on the anhydrous basis.

Category. Anticancer.

Description. An orange-red powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *epirubicin hydrochloride IPRS* or with the reference spectrum of epirubicin hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 0.5 ml of *nitric acid*, add 0.5 ml of *water* and heat over a flame for 2 minutes. Allow to cool and add 0.5 ml of *silver nitrate solution*. A white precipitate is formed.

Tests

pH (2.4.24). 4.0 to 5.5, determined in 0.5 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE —Allow the solutions to stand for 3 hours before use.

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *epirubicin hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v each of *epirubicin hydrochloride IPRS* and *doxorubicin hydrochloride IPRS* in the mobile phase.

Reference solution (c). Dissolve 10 mg of *doxorubicin hydrochloride IPRS* in a mixture of 5 ml of *water* and 5 ml of *orthophosphoric acid*. Allow to stand for 30 minutes. Adjusted to pH 2.6 with 8 per cent w/v solution of *sodium hydroxide*. Add 15 ml of *acetonitrile*, 10 ml of *methanol* and mix.

Reference solution (d). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with trimethylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 54 volumes of a buffer solution prepared by dissolving 3.7 g of *sodium lauryl sulphate* and 28 ml of *dilute phosphoric acid* in 1000 ml of *water*, 17 volumes of *methanol* and 29 volumes of *acetonitrile*,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Epirubicin impurity A[1]	0.3	0.7
Epirubicin impurity B[2]	0.4	—
Epirubicin impurity C[3]	0.8	—
Epirubicin (Retention time: about 9.5 minutes)	1.0	—
Epirubicin impurity D[4]	1.5	—
Epirubicin impurity E[5]	1.1	—
Epirubicin impurity F[6]	1.7	—
Epirubicin impurity G[7]	2.1	—

[1]R = OH: (8*S*,10*S*)-6,8,10,11-tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (doxorubicinone),

[2]R = H: (8 *S*,10*S*)-8-acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicinone),

[3](8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-*L-lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (doxorubicin),

[4](8*S*,10*S*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-*L-lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicin),

[5](8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-*L-lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-[(1*RS*)-1-hydroxyethyl]-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (dihydrodaunorubicin),

[6](8*S*,10*S*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-*L-arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (*epi*-daunorubicin),

8,82-[(2*R*,4*R*)-4-hydroxy-2-(hydroxymethyl)-1,3-dioxolan-2,4-diyl]bis[(8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-*L-arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione] (epirubicin dimer).

Run the chromatogram 3.5 times the retention time of epirubicin.

Inject reference solution (c). In the chromatogram, use the second most abundant peak to identify impurity A.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to impurity C and epirubicin is not less than 2.0.

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to epirubicin impurities A and C are not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent). The sum of areas of all secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Acetone (5.4). Not more than 1.5 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{27}H_{30}ClNO_{11}$.

Epirubicin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.1 Endotoxin Unit per mg of epirubicin hydrochloride.

Storage. Store protected from light and moisture, at a temperature between 2° and 8°. If the substance is sterile, store in a sterile, tamper-proof container.

Epirubicin Injection

Epirubicin Hydrochloride Injection

Epirubicin Injection is a sterile solution of Epirubicin Hydrochloride in Water for Injections.

Epirubicin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of epirubicin hydrochloride, $C_{27}H_{30}ClNO_{11}$.

Usual strengths. 2 mg per ml.

Identification

A. Dilute a volume of injection to obtain 0.001 per cent w/v of epirubicin hydrochloride in *water*. When examined in the range 220 nm to 350 nm (2.4.7), the solution shows an absorption maximum at 233, 253 and 292 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 2.5 to 4.0.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE —Allow the solutions to stand for 3 hours before use.

Test solution. Dilute a volume of the injection with sufficient mobile phase to produce a solution containing 0.1 per cent w/v of Epirubicin Hydrochloride.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). A 0.01 per cent w/v each of *epirubicin hydrochloride IPRS* and *doxorubicin hydrochloride IPRS* in the mobile phase.

Reference solution (c). Dissolve 10 mg of *doxorubicin hydrochloride IPRS* in a mixture of 5 ml of *water* and 5 ml of *orthophosphoric acid* and allow to stand for 30 minutes. Adjust the pH of the solution to 2.6 with 8 per cent w/v solution of *sodium hydroxide*, add 15 ml of *acetonitrile* and 10 ml of *methanol* and mix (generation of impurity A).

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with trimethylsilane bonded to porous silica (6 µm) (Such as Zorbax TMS),
- column temperature: 35°,
- mobile phase: a mixture of 54 volumes of a buffer solution prepared by dissolving 3.7 g of *sodium lauryl sulphate* and 28 ml of *1M orthophosphoric acid* in 1000 ml of *water*, 17 volumes of *methanol* and 29 volumes of *acetonitrile*,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Epirubicin impurity A[1]	0.3	0.7
Epirubicin impurity B[2]	0.4	—
Epirubicin impurity C[3]	0.8	—
Epirubicin (Retention time: about 9.5 minutes)	1.0	—
Epirubicin impurity D[4]	1.5	—
Epirubicin impurity E[5]	1.1	—
Epirubicin impurity F[6]	1.7	—
Epirubicin impurity G[7]	2.1	—

[1]R = OH:(8*S*,10*S*)-6,8,10,11-tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (doxorubicinone),

[2]R = H: (8 *S*,10*S*)-8-acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicinone),

[3](8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-*L-lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (doxorubicin),

[4](8*S*,10*S*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-*L-lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicin),

[5](8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-*L-lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-[(1*RS*)-1-hydroxyethyl]-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (dihydrodaunorubicin),

[6](8*S*,10*S*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-*L-arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (*epi*-daunorubicin),

[7]8,82-[(2*R*,4*R*)-4-hydroxy-2-(hydroxymethyl)-1,3-dioxolan-2,4-diyl]bis[(8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-*L-arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione] (epirubicin dimer).

Identify any peak in the chromatogram obtained with test solution corresponding to epirubicin impurity A using the second most abundant peak in the chromatogram obtained with reference solution (c) and multiply the area of this peak by the corresponding correction factor of 0.7.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to epirubicin and doxorubicin is not less than 2.0.

Inject reference solution (a) and the test solution. Run the chromatogram 3.5 times the retention time of epirubicin. In the chromatogram obtained with the test solution, the area of peak corresponding to epirubicin impurities A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). The area of peak corresponding to epirubicin impurities C is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of areas of all secondary peaks is not more than four times the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 2.2 Endotoxin Unit per ml. A solution containing 2 mg per ml of Epirubicin Hydrochloride.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

NOTE — Allow the solutions to stand for 3 hours before use.

Test solution. Dilute a volume of the injection with sufficient mobile phase to produce a solution containing 0.1 per cent w/v of Epirubicin Hydrochloride.

Reference solution (a). A 0.1 per cent w/v solution of *epirubicin hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v each of *epirubicin hydrochloride IPRS* and *doxorubicin hydrochloride IPRS* in the mobile phase.

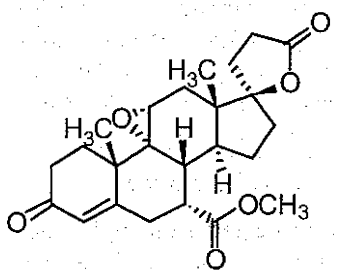
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to epirubicin and doxorubicin is not less than 2.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{27}H_{30}ClNO_{11}$ in the injection.

Storage. Store at a temperature between 2° and 8°.

Eplerenone



$C_{24}H_{30}O_6$ Mol. Wt. 414.5

Eplerenone is 9α,11-Epoxy-7α-methoxycarbonyl-3-oxo-17α-pregn-4-ene-21,17-carbolactone.

Eplerenone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{24}H_{30}O_6$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white to off-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *eplerenone IPRS* or with the reference spectrum of eplerenone.

Tests

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay with the following modification.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *eplerenone IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *10 mM ammonium acetate*, adjusted to pH 7.4 and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{30}O_6$.

Eplerenone Tablets

Eplerenone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of eplerenone, $C_{24}H_{30}O_6$.

Usual strengths. 25 mg; 50 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *eplerenone IPRS* in 5 ml of *methanol* and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of 0.05 per cent v/v solution of *orthophosphoric acid* and 30 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{30}O_6$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{24}H_{30}O_6$.

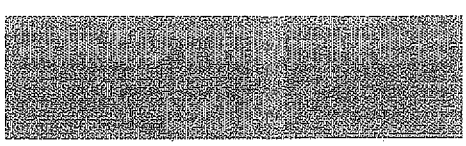
Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *water* and 80 volumes of *acetonitrile*.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Eplerenone in 70 ml of the solvent mixture, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture and filter.

Reference solution. A 0.0005 per cent w/v solution of *eplerenone IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),



– mobile phase: A. 0.05 per cent v/v solution of *orthophosphoric acid*,
B. *acetonitrile*,
– a gradient programme using the conditions given below,
– flow rate: 1 ml per minute,
– spectrophotometer set at 240 nm,
– injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
10	80	20
35	25	75
40	80	20
45	80	20

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *water* and 80 volumes of *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 100 mg of Eplerenone in the solvent mixture, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture and filter. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *eplerenone IPRS* in the solvent mixture.

Use the chromatographic system as described under Dissolution.

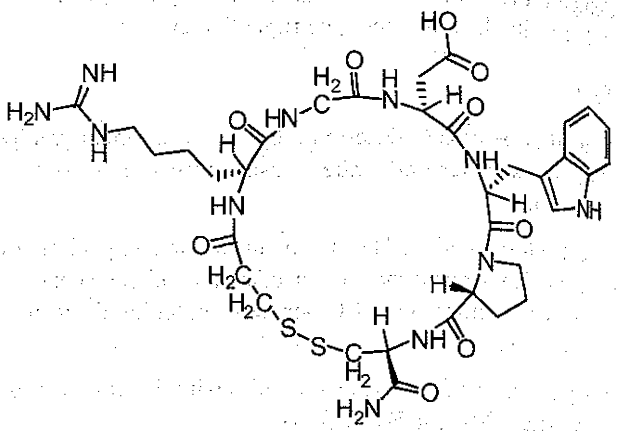
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{30}O_6$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Eptifibatide



$C_{35}H_{49}N_{11}O_9S_2$ Mol. Wt. 832

Eptifibatide is *S*1,*S*6-Cyclo[*N*6-carbamimidoyl-*N*2-(3-sulfanylpropanoyl)-*L*-lysylglycyl-*L*-α-aspartyl-*L*-tryptophyl-*L*-prolyl-*L*-cysteinamide].

Eptifibatide contains not less than 96.0 per cent and not more than the equivalent of 103.0 per cent of $C_{35}H_{49}N_{11}O_9S_2$, calculated on the anhydrous and acetic acid free basis.

Category. Antiplatelet.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *eptifibatide IPRS* or with the reference spectrum of eptifibatide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Light absorption. Absorbance of 1.0 per cent w/v solution in *acetic acid* at 420 nm (2.4.6) is not more than 0.05.

Specific optical rotation (2.4.22). –57.0° to –50.0°, determined in a 1.0 per cent w/v solution in *1.0 per cent v/v acetic acid*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution. A 0.02 per cent w/v solution of *eptifibatide IPRS* in *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 85 volumes of 0.25 per cent w/v solution of *orthophosphoric acid* in *water*, adjusted to pH 3.0 with *triethylamine* and 15 volumes of *acetonitrile*,
- flow rate: 1 ml per minute.
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. The area of any secondary peak is not more than 1.5 per cent and the sum of areas of all the secondary peaks is not more than 2.5 per cent, calculated by area normalization.

Acetic Acid. Not more than 8.0 per cent.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 95 volumes of mobile phase A and 5 volumes of *methanol*.

Test solution. Dissolve 4 mg of the substance under examination in the solvent mixture and dilute to 2.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *glacial acetic acid IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.07 per cent v/v solution of *orthophosphoric acid* in *water*, adjusted to pH 3.0 with *1 M sodium hydroxide*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute.
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0.01	95	5
10	50	50
20	50	50
21	50	50
30	95	5
35	95	5

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

The retention time of acetic acid is about 3.9 minutes.

Inject the reference solution and the test solution.

Calculate the content of acetic acid.

Water (2.3.43). Not more than 9.0 per cent, using Method 3.

Bacterial endotoxins (2.2.3). Not more than 10 Endotoxin Units per mg of eptifibatide.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 2 mg of the substance under examination in *water* and dilute to 10.0 ml with *water*.

Reference solution. A 0.02 per cent w/v solution of *eptifibatide IPRS* in *water*.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{35}H_{49}N_{11}O_9S_2$.

Eptifibatide Injection

Eptifibatide Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of eptifibatide, $C_{35}H_{49}N_{11}O_9S_2$.

Description. A clear, colourless solution.

Usual strength. 0.75 mg per ml; 2 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

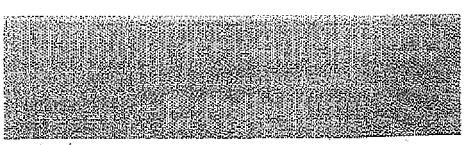
pH (2.4.24). 5.0 to 5.5.

Light absorption. Not more than 0.1, determined on 0.2 per cent w/v solution at 420 nm (2.4.7).

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay with the following modifications.

Test solution. Dilute the injection containing 8 mg of Eptifibatide to 10.0 ml with *water*

Inject the test solution. The area of any secondary peak is not more than 2.0 per cent and the sum of areas of all the secondary peaks is not more than 5.0 per cent, calculated by area normalization.



Bacterial endotoxins (2.2.3). Not more than 10 Endotoxin Units per mg of eptifibatide.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the injection to obtain a solution containing 0.024 per cent w/v of Eptifibatide in *water*.

Reference solution. A 0.024 per cent w/v solution of *eptifibatide IPRS* in *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 85 volumes of buffer solution prepared by dissolving 2.45 g of *orthophosphoric acid* in 1000 ml of *water*, adjusted to pH 3.0 with *triethylamine* and 15 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3600 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

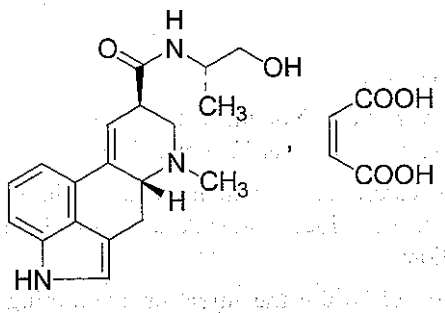
Inject the reference solution and the test solution.

Calculate the content of $C_{35}H_{49}N_{11}O_9S_2$ in the injection.

Storage. Store protected from light.

Ergometrine Maleate

Ergonovine Maleate



$C_{19}H_{23}N_3O_2,C_4H_4O_4$ Mol.Wt. 441.5

Ergometrine Maleate is 9,10-didehydro-*N*-[(*S*)-2-hydroxy-1-methylethyl]-6-methylergoline-8β-carboxamide hydrogen maleate.

Ergometrine Maleate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{23}N_3O_2,C_4H_4O_4$, calculated on the dried basis.

Category. Uterine stimulant.

Description. A white or faintly yellow, crystalline powder. It is affected by light.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, D and E may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ergometrine maleate IPRS* or with the reference spectrum of ergometrine maleate.

B. Dissolve 30 mg in sufficient *0.01 M hydrochloric acid* to produce 100 ml and dilute 10 ml of the solution to 100 ml with the same solvent. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 311 nm and a minimum at 265 nm to 272 nm; absorbance at 311 nm, 0.52 to 0.58.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g, without heating and protected from light, in sufficient *carbon dioxide-free water* to produce 10 ml (solution A). To 0.1 ml of solution A add 1 ml of *glacial acetic acid*, 1 drop of *ferric chloride solution* and 1 ml of *orthophosphoric acid* and heat on a water-bath at 80°; a blue or violet colour is produced after about 10 minutes.

E. To 1 ml of a 0.01 per cent w/v solution, add 2 ml of *4-dimethylaminobenzaldehyde reagent*; a deep blue colour is produced after about 10 minutes.

Tests

Appearance of solution. Solution A is clear (2.4.1), and not more intensely coloured than reference solution YS5 or BYS5 (2.4.1).

pH (2.4.24). 3.6 to 4.4, determined in solution A.

Specific optical rotation (2.4.22). +50.0° to +56.0°, determined in solution A.

Related substances. *Carry out the following operations as rapidly as possible, protected from light.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 75 volumes of *chloroform*, 25 volumes of *methanol* and 3 volumes of *water*.

NOTE—Prepare the following solutions freshly.

Solvent mixture. A mixture of 1 volume of *strong ammonia solution* and 9 volumes of *ethanol (80 per cent)*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml with solvent mixture.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *ergometrine maleate IPRS* in the solvent mixture.

Reference solution (b). A 0.01 per cent w/v solution of *ergometrine maleate IPRS* in the solvent mixture.

Reference solution (c). A 0.005 per cent w/v solution of *ergometrine maleate IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 14 cm. Dry the plate in a current of cold air and spray with *4-dimethylaminobenzaldehyde reagent*. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (c).

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 0.2 g by drying in an oven at 80° at a pressure not exceeding 2.7 kPa for 2 hours.

Assay. Weigh 0.15 g and dissolve in 40 ml of *anhydrous glacial acetic acid*. Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.05 M perchloric acid* is equivalent to 0.02207 g of $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

Storage. Store protected from light in a refrigerator (2° to 8°).

Ergometrine Injection

Ergometrine Maleate Injection; Ergonovine Injection, Ergonovine Maleate Injection

Ergometrine Injection is a sterile solution of Ergometrine Maleate in Water for Injections containing suitable stabilising agents.

Ergometrine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergometrine maleate, $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

Usual strength. 500 µg per ml.

Description. A clear, colourless or faintly yellow solution.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

B. Exhibits a blue fluorescence.

C. To a volume containing 0.1 mg of Ergometrine Maleate, add 0.5 ml of *water* and 2 ml of *4-dimethylaminobenzaldehyde reagent*; a deep blue colour is produced after 10 minutes.

Tests

pH (2.4.24). 2.7 to 3.5.

Related substances. *Carry out the following procedure in subdued light and protect from light any solutions not used immediately.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* slurried with *0.1 M sodium hydroxide*.

Mobile phase. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Evaporate a volume of the injection containing 1 mg of Ergometrine Maleate to dryness at 20° at a pressure not exceeding 2 kPa and dissolve the residue in 0.25 ml of methanol.

Reference solution (a). A 0.4 per cent w/v solution of *ergometrine maleate IPRS* in *methanol*.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with *methanol*.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 10.0 ml with *methanol*.

Reference solution (d). Dilute 5.0 ml of reference solution (c) to 10.0 ml with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. Assess the intensities of any secondary spots in the chromatogram obtained with the test solution by reference to the spots in the chromatograms obtained with reference solution (b), (c) and (d). The total of the intensities so assessed does not exceed 10 per cent of the intensity of the principal spot.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *Carry out the following procedure protected from light.*

Dilute a suitable volume, accurately measured, of the injection with sufficient *water* to produce a solution containing 0.004 per cent w/v of Ergometrine Maleate. To 3.0 ml add 6.0 ml

of *4-dimethylaminobenzaldehyde reagent*, mix, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time prepare solution B in the same manner but using 3.0 ml of a 0.004 per cent w/v solution of *ergometrine maleate IPRS* and beginning at the words "add 6.0 ml.....". Measure the absorbance of solution B at the maximum at about 545 nm (2.4.7), using as the blank a solution prepared by mixing 6.0 ml of *4-dimethylaminobenzaldehyde solution* and 3.0 ml of *water*. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

Storage. Store protected from light in single dose containers in a refrigerator (2° to 8°).

Ergometrine Tablets

Ergometrine Maleate Tablets; Ergonovine Tablets; Ergonovine Maleate Tablets

Ergometrine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergometrine maleate, $C_{19}H_{23}N_3O_2,C_4H_4O_4$.

Usual strengths. 250 µg; 500 µg.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

B. Extract a quantity of the powdered tablets containing 2 mg of Ergometrine Maleate with 20 ml of *water*, filter and wash the residue with sufficient *water* to produce 20 ml. The solution exhibits a blue fluorescence.

C. To 2 ml of the solution obtained in test B add 4 ml of *4-dimethylaminobenzaldehyde reagent*; a deep blue colour is produced after about 10 minutes.

Tests

Related substances. *Carry out the following procedure in subdued light and protect from light any solutions not used immediately.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* slurried with *0.1 M sodium hydroxide*.

Mobile phase. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Triturate a quantity of the powdered tablets containing about 1 mg of Ergometrine Maleate with 0.2 ml of a 1 per cent w/v solution of *domiphen bromide*, add 2 ml of *methanol*, centrifuge and remove the supernatant liquid. Extract the residue with two quantities, each of 1 ml, of *methanol*, evaporate the combined extracts to dryness at 20° at a pressure not exceeding 2 kPa and dissolve the residue in 0.25 ml of *methanol*, centrifuge if necessary.

Reference solution (a). A 0.4 per cent w/v solution of *ergometrine maleate IPRS* in *methanol*.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with *methanol*.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 10.0 ml with *methanol*.

Reference solution (d). Dilute 5.0 ml of reference solution (c) to 10.0 ml with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. Assess the intensities of any secondary spots in the chromatogram obtained with the test solution by reference to the spots in the chromatograms obtained with reference solution (b), (c) and (d). The total of the intensities so assessed does not exceed 10 per cent of the intensity of the principal spot.

Uniformity of content. Complies with the test stated under Tablets.

NOTE—Protect the solutions from light throughout the test.

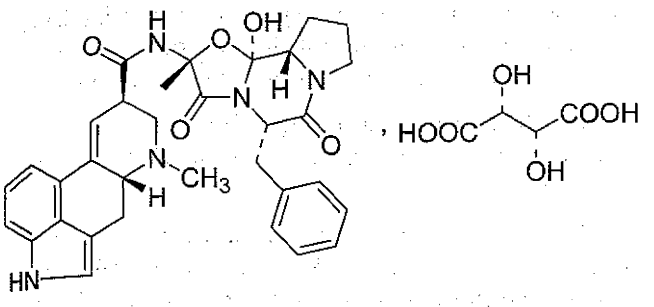
To one tablet add 10.0 ml of a 1 per cent w/v solution of *tartaric acid*, shake for 30 minutes, centrifuge and use the supernatant liquid. Dilute a suitable volume, accurately measured, with sufficient *water* to produce a solution containing 0.004 per cent w/v of Ergometrine Maleate. To 3.0 ml add 6.0 ml of *4-dimethylaminobenzaldehyde solution*, mix, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time prepare solution B in the same manner but using 3.0 ml of a 0.004 per cent w/v solution of *ergometrine maleate IPRS* and beginning at the words "add 6.0 ml...." Measure the absorbance of solution B at the maximum at about 545 nm (2.4.7), using as the blank a solution prepared by mixing 6.0 ml of *4-dimethylaminobenzaldehyde reagent* and 3.0 ml of *water*. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 2 mg of Ergometrine Maleate, shake with 50.0 ml of a 1 per cent w/v solution of *tartaric acid* for 30 minutes, centrifuge and use the supernatant liquid. Carry out the procedure described under Uniformity of content beginning at the words "To 3.0 ml add 6 ml.....".

Storage. Store protected from light.

Ergotamine Tartrate



$(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ Mol. Wt. 1313.4

Ergotamine Tartrate is (5'*S*)-12'-hydroxy-2'-methyl-3',6',18-trioxo-5-benzylergotaman tartrate.

Ergotamine Tartrate contains not less than 98.0 per cent and not more than 101.0 per cent of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$, calculated on the dried basis.

Category. Antimigraine.

Description. Colourless crystals, or a white or almost white, crystalline powder; slightly hygroscopic. It may contain two molecular equivalents of methanol of crystallisation.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Before triturating with *potassium bromide IR* during preparation of the disc, triturate first with 0.2 ml of *methanol*. Compare the spectrum with that obtained with *ergotamine tartrate IPRS* or with the reference spectrum of ergotamine tartrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent solution in *0.01 M hydrochloric acid* shows an absorption maximum at 311 nm to 321 nm and a minimum at 265 nm to 275 nm; absorbance at the maximum, 0.59 to 0.64, calculated on the dried basis.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a) when examined for not more than 1 minute under ultraviolet light at 365 nm or when examined in daylight after spraying with *ethanolic 4-dimethylaminobenzaldehyde solution*.

D. Dissolve 1 mg in a mixture of 5 ml of *glacial acetic acid* and 5 ml of *ethyl acetate*. To 1 ml of the solution add 1 ml of *sulphuric acid*, with continuous shaking and cooling; a blue colour with a red tinge develops. Add 0.1 ml of *ferric chloride test solution* previously diluted with an equal volume of *water*; the red tinge becomes less apparent and the blue colour more pronounced.

E. Dissolve 1 mg in 5 ml of a 1 per cent w/v solution of *tartaric acid*. To 1 ml of the solution add slowly 3 ml of *4-dimethylaminobenzaldehyde solution* and mix; a deep blue colour is produced.

Tests

NOTE—Carry out the following tests as rapidly as possible, protected from light.

Appearance of solution. Mix 50 mg with 25 mg of *tartaric acid* and dissolve in 20 ml of *water*. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 4.0 to 6.0, determined in a 0.25 per cent w/v suspension.

Specific optical rotation (2.4.22). The specific optical rotation of the ergotamine base, checked for purity by the method given below, is −165° to −154°, determined by the following method. Dissolve 0.4 g in 40 ml of a 1 per cent w/v solution of *tartaric acid*, cautiously add 0.5 g of *sodium bicarbonate* in small portions and mix well. Wash 100 ml of *chloroform* by shaking with 5 quantities, each of 50 ml, of *water* and extract the solution of the substance under examination with 4 quantities, each of 10 ml, of the washed chloroform. Filter the combined chloroform extracts through a small filter moistened with the washed chloroform, dilute to 50 ml with the same solvent and measure the optical rotation.

To 25 ml of the chloroform solution add 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.05 M perchloric acid* is equivalent to 0.02908 g of ergotamine base, $C_{33}H_{35}N_5O_5$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 70 volumes of *ether*, 15 volumes of *dimethylformamide*, 10 volumes of *chloroform* and 5 volumes of *ethanol*.

NOTE—Prepare the following solutions immediately before use in the order stated.

Solvent mixture. 9 volumes of *chloroform* and 1 volume of *methanol*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10.0 ml with the solvent mixture.

Test solution (b). Dilute 5.0 ml of test solution (a) to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *ergotamine tartrate IPRS* in the solvent mixture.

Reference solution (b). A 0.015 per cent w/v solution of *ergotamine tartrate IPRS* in the solvent mixture.

Reference solution (c). A 0.005 per cent w/v solution of *ergotamine tartrate IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Immediately after application expose the plate to an atmosphere saturated with ammonia vapour for exactly 20 seconds, dry the plate at the line of application in a current of cold air and immediately start developing the chromatogram, allowing the mobile phase to rise 17 cm. Dry the plate in a current of dry air for 2 minutes and examine under ultraviolet light at 365 nm for not more than 1 minute. Spray abundantly with *ethanolic 4-dimethylaminobenzaldehyde solution* and dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (c).

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 0.1 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 6 hours.

Assay. Weigh 0.2 g and dissolve in 40 ml of *anhydrous glacial acetic acid* Titrate with *0.05 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.05 M perchloric acid* is equivalent to 0.03284 g of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$.

Storage. Store protected from light in sealed glass containers, in a refrigerator (2° to 8°).

Ergotamine Injection

Ergotamine Tartrate Injection

Ergotamine Injection is a sterile solution of Ergotamine Tartrate in Water for Injection containing Ethanol (95 per cent), Glycerin and sufficient Tartaric Acid to adjust the pH of the solution to 3.3.

Ergotamine Injection contains a quantity of total alkaloids, calculated as $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$, equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergotamine tartrate.

Usual strength. 500 µg per ml.

Description. A clear, colourless or almost colourless solution.

Identification

A. In the test for Ergot alkaloids and related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that due to ergotamine in the chromatogram obtained with the reference solution.

B. To a volume containing 0.2 mg of Ergotamine Tartrate add 1 ml of *4-dimethylaminobenzaldehyde solution*; a deep blue colour is produced.

C. Mix a volume containing about 2 mg of Ergotamine Tartrate with 2 ml of *dilute sulphuric acid*, dissolve a few mg of *magnesium powder* in the solution and add 25 mg of *resorcinol*. Shake to dissolve, carefully add 2 ml of *sulphuric acid* down the inside of the tube and warm gently; a red ring forms at the interface of the two liquid layers and spreads throughout the lower layer.

Tests

NOTE—Carry out the following tests as rapidly as possible, protected from light.

pH (2.4.24). 2.8 to 3.8.

Ergot alkaloids and related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* slurried with *0.1 M sodium hydroxide*.

Mobile phase. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Add sufficient of a 10 per cent w/v solution of *sodium bicarbonate* to a volume of the injection containing about 5 mg of Ergotamine Tartrate to make it distinctly alkaline to litmus paper. Extract with five quantities, each of 10 ml, of *chloroform*, filter the extracts through a small double filter paper, wash the filter with *chloroform*, evaporate the combined filtrates and washings to dryness at 20° at a pressure of about 1.5 kPa and dissolve the residue in 1 ml of a mixture of equal volumes of *chloroform* and *methanol*.

Reference solution. Dissolve 5 mg of *ergotamine tartrate IPRS* in 10 ml of a 1 per cent w/v solution of *tartaric acid* and complete the preparation described for the test solution beginning at the words "Extract with five quantities...".

Apply without delay, to the plate 20 µl of the test solution and 14 µl, 10 µl, 7 µl and 2 µl of the reference solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. The chromatogram obtained with the test solution shows two principal spots, corresponding to ergotamine and, of higher R_f value, ergotaminine; a spot between the two principal spots and a number of spots of lower R_f values may also be seen. Compare the chromatogram obtained with the test solution with the chromatograms obtained with the reference solution. The spot corresponding to ergotaminine is not larger or more intense than the spot

corresponding to ergotamine obtained with 7 µl of the reference solution. The spot corresponding to ergotamine is not smaller or less intense than the spot corresponding to ergotamine obtained with 10 µl of the reference solution and is not larger or more intense than the spot corresponding to ergotamine obtained with 14 µl of the reference solution, corresponding to not less than 50 per cent and not more than 70 per cent of ergotamine tartrate. Any other spots are not larger or more intense than the spot corresponding to ergotamine obtained with 2 µl of the reference solution.

Bacterial endotoxins (2.2.3). Not more than 357.0 Endotoxin Units per mg of ergotamine.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume add sufficient of a 0.25 per cent w/v solution of *tartaric acid* to produce a solution containing about 0.005 per cent w/v of Ergotamine Tartrate. Mix 3.0 ml of the solution with 6.0 ml of *4-dimethylaminobenzaldehyde solution*, cool to room temperature and allow to stand for 30 minutes (solution A). At the same time, mix 3.0 ml of a 0.003 per cent w/v solution of *ergometrine maleate IPRS* in a 0.25 per cent w/v solution of *tartaric acid* with 6.0 ml of *4-dimethylaminobenzaldehyde solution*, cool to room temperature and allow to stand for 30 minutes (solution B). Prepare solution C by mixing 3.0 ml of a 0.25 per cent w/v solution of *tartaric acid* with 6.0 ml of *4-dimethylaminobenzaldehyde solution*. Measure the absorbance of solution B at 545 nm (2.4.7), using solution C as the blank. Without delay replace solution B with solution A, using the same cell, and measure the absorbance of solution A at the same wavelength. Calculate the content of total alkaloids as $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$ from the absorbances obtained.

1 mg of *ergometrine maleate IPRS* is equivalent to 1.488 mg of $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$.

Storage. Store protected from light in single dose glass containers.

Ergotamine Tablets

Ergotamine Tartrate Tablets

Ergotamine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ergotamine tartrate, $(C_{33}H_{35}N_5O_5)_2,C_4H_6O_6$.

Usual strength. 1 mg.

Identification

A. Triturate a quantity of the powdered tablets containing about 5 mg of Ergotamine Tartrate with 10 ml of *light petroleum (40° to 60°)*, allow to settle and discard the petroleum extract. To the residue add 10 ml of *chloroform* saturated with *strong ammonia solution*, triturate, filter and evaporate the filtrate to dryness on a water-bath. The residue so obtained complies with the following tests.

Dissolve 1 mg in a mixture of 5 ml of *glacial acetic acid* and 5 ml of *ethyl acetate*. To 1 ml of the solution add 1 ml of *sulphuric acid*, with continuous shaking and cooling; a blue colour with a red tinge develops. Add 0.1 ml of *ferric chloride test solution* previously diluted with an equal volume of *water*; the red tinge becomes less apparent and the blue colour more pronounced.

B. Dissolve 1 mg in 5 ml of a 1 per cent w/v solution of *tartaric acid*. To 1 ml of the solution add slowly 3 ml of *4-dimethylaminobenzaldehyde solution* and mix; a deep blue colour is produced.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

NOTE—Carry out the following tests as rapidly as possible, protected from light.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 1000 ml of 1 per cent w/v solution of *tartaric acid* in *water*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the fluorescence intensities of the filtrate, dilute suitably if necessary with the medium, using the maximum excitation wavelength at 327 nm and the maximum emission wavelength at 427 nm (2.4.5). Calculate the content of $(C_{33}H_{35}N_2O)_2,C_4H_6O_6$ in the medium by comparing the fluorescence intensities obtained from a solution of a known concentration of *ergotamine tartrate IPRS* in dissolution medium.

Q. Not less than 75 per cent of the stated amount of $(C_{33}H_{35}N_2O)_2,C_4H_6O_6$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 70 volumes of *ether*, 15 volumes of *dimethylformamide*, 10 volumes of *chloroform* and 5 volumes of *ethanol*.

NOTE—Prepare the following solutions immediately before use in the order stated.

Test solution. Extract a quantity of the powdered tablets containing 1 mg of Ergotamine Tartrate with 2 ml of a mixture of equal volumes of *chloroform* and *methanol* and centrifuge.

Remove the supernatant liquid, extract the residue with two quantities, each of 1 ml, of the solvent mixture, evaporate the combined extracts to dryness at 20° at a pressure of 2 kPa and dissolve the residue in 0.25 ml of a mixture of equal volumes of *chloroform* and *methanol*; centrifuge if necessary.

Reference solution (a). A 0.4 per cent w/v solution of *ergotamine tartrate IPRS* in the same solvent mixture.

Reference solution (b). A 0.04 per cent w/v solution of *ergotamine tartrate IPRS* in the same solvent mixture.

Reference solution (c). A 0.02 per cent w/v solution of *ergotamine tartrate IPRS* in the same solvent mixture.

Reference solution (d). A 0.01 per cent w/v solution of *ergotamine tartrate IPRS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. Immediately after application expose the plate to an atmosphere saturated with ammonia vapour for exactly 20 seconds, dry the plate at the line of application in a current of cold air and immediately start developing the chromatogram, allowing the mobile phase to rise 17 cm. Dry the plate in air and examine under ultraviolet light at 365 nm. Assess the intensity of any secondary spots in the chromatogram obtained with the test solution by reference to the spots in the chromatograms obtained with reference solution (a), (b) and (c) and the sum of the intensities so assessed in the chromatogram obtained with the test solution should not exceed 10 per cent of the intensity of the principal spot in the chromatogram obtained with the test solution. In addition, any single secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (d).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse one tablet in 5.0 ml of internal standard solution and sufficient quantity of the solvent mixture, with the aid of ultrasound for 10 minutes and dilute with the solvent mixture to obtain a solution containing 0.002 per cent w/v of Ergotamine Tartrate.

Inject the reference solution and the test solution.

Calculate the content of $(C_{33}H_{35}N_2O)_2,C_4H_6O_6$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 55 volumes of *acetonitrile* and 45 volumes of *water*.

Internal standard solution: A 0.016 per cent w/v solution of *ergometrine maleate* in the solvent mixture.

Test solution. Disperse a quantity of intact tablets containing 10 mg of Ergotamine Tartrate in 50.0 ml of internal standard solution, add 300 ml of the solvent mixture, with the aid of ultrasound for 10 minutes and dilute to 500.0 ml with the solvent mixture, filter.

Reference solution. A 0.02 per cent w/v solution of *ergotamine tartrate IPRS* in the solvent mixture. To 5.0 ml of the solution, add 5.0 ml of internal standard solution and dilute to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *0.01 M monobasic potassium phosphate* and 55 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The relative retention time with respect to ergotamine tartrate for ergometrine maleate is about 0.7.

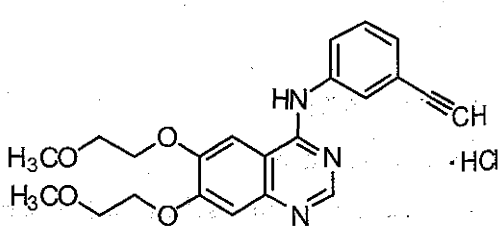
Inject the reference solution. The test is not valid unless the resolution between the peaks due to ergometrine maleate and ergotamine tartrate is not less than 3.0, the column efficiency is not less than 3000 theoretical plates the tailing factor is not more than 2.0 per cent and the relative standard deviation for replicate injections is not more than 2.0 per cent for ergotamine tartrate.

Inject the reference solution and the test solution.

Calculate the content of $(C_{33}H_{35}N_2O)_2,C_4H_6O_6$ in the tablets.

Storage. Store protected from light at a temperature not exceeding 30°.

Erlotinib Hydrochloride



$C_{22}H_{23}N_3O_4,HCl$ Mol. Wt. 429.9

Erlotinib Hydrochloride is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine hydrochloride.

Erlotinib Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{23}N_3O_4.HCl$, calculated on anhydrous basis.

Category. Anticancer.

Description. An off-white to pale yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *erlotinib hydrochloride IPRS* or with the reference spectrum of erlotinib hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Solvent mixture. 40 volumes of mobile phase A and 60 volumes of mobile phase B.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.00025 per cent w/v solution of *erlotinib hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3 µm),
- column temperature: 50°,
- mobile phase: A. *0.02 M sodium dihydrogen phosphate* in 0.1 per cent v/v *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
2	85	15
20	75	25
25	40	60
30	40	60
30.1	85	15
35	85	15

The retention time of the principal peak is about 19 minutes.

Inject the reference solution. The test is not valid unless the the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 10.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *erlotinib hydrochloride IPRS* in the solvent mixture.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{23}N_3O_4.HCl$.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Erlotinib Tablets

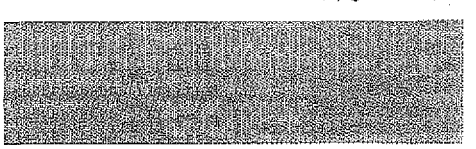
Erlotinib Hydrochloride Tablets

Erlotinib Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of erlotinib, $C_{22}H_{23}N_3O_4$.

Usual strengths. 25mg; 100 mg; 150 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.



Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 1000 ml of 1 per cent w/v of *sodium lauryl sulphate* in *0.1 M hydrochloric acid,*

Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate if necessary, with the mobile phase.

Reference solution. Dissolve a quantity of *erlotinib hydrochloride IPRS* in sufficient amount of *methanol* and dilute with the dissolution medium to obtain a 0.016 per cent w/v solution. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3 µm),
- column temperature: 50°,
- mobile phase: a mixture of 60 volumes of a solution containing *0.02 M sodium dihydrogen orthophosphate* in 0.1 per cent v/v *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid* and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of erlotinib, $C_{22}H_{23}N_3O_4$.

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{23}N_3O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of mobile phase A and 60 volumes of mobile phase B.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of Erlotinib with 30 ml of solvent mixture with the aid of ultrasound for 20 minutes and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.00055 per cent w/v of *erlotinib hydrochloride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3 µm),
- column temperature: 50°,
- mobile phase: A. *0.02 M sodium dihydrogen orthophosphate* in 0.1 per cent v/v *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid*,

B. *acetonitrile,*

- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
2	85	15
20	75	25
25	40	60
45	40	60
45.1	85	15
55	85	15

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of mobile phase A and 60 volumes of mobile phase B.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of erlotinib in 30 ml of the solvent mixture with the aid of ultrasound for 20 minutes and dilute to 100.0 ml with the solvent mixture, filter. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *erlotinib hydrochloride IPRS* in the solvent mixture.

Use chromatographic system as described under Related substances, using the following gradient programme.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
2	85	15
20	75	25
25	40	60
30	40	60
30.1	85	15
35	85	15

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

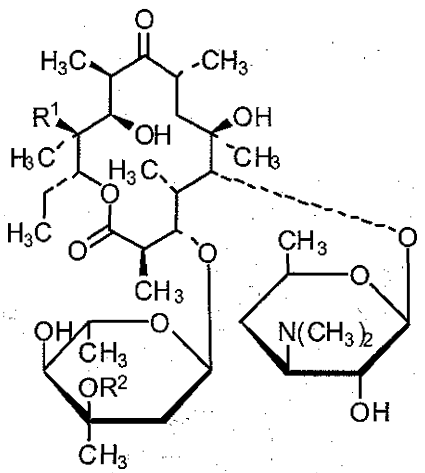
Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{23}N_3O_4$ in the tablets.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of erlotinib.

Erythromycin



$C_{37}H_{67}NO_{13}$ Mol. Wt. 733.9

Erythromycin is a mixture of macrolide antibiotics consisting largely of erythromycin A, (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-4-[(2,6- dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13- trihydroxy-3,5,7,9,11,13-hexamethyl-6-[(3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl)-oxy] oxacyclotetradecane-2,10-dione, it is produced by the growth of certain strains of *Streptomyces erythreus*.

Erythromycin has a potency not less than 920 Units per mg, calculated on the anhydrous basis.

Category. Antibacterial.

Description. Colourless or slightly yellow crystals or a white or slightly yellow powder; slightly hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *erythromycin IPRS* or with the reference spectrum of erythromycin.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. The upper layer obtained by shaking together 45 volumes of *ethyl acetate*, 40 volumes of a 15 per cent w/v solution of *ammonium acetate* previously adjusted to pH 9.6 with *10 M ammonia* and 20 volumes of *2-propanol* and allowing to separate.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.1 per cent w/v solution of *erythromycin IPRS* in *methanol*.

Reference solution (b). A 0.2 per cent w/v solution of *spiramycin IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and is different in position and colour from the spots in the chromatogram obtained with reference solution (b).

C. To about 5 mg add 5 ml of a 0.02 per cent w/v solution of *xanthydrol* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of *5 M acetic acid* and heat on a water-bath; a red colour is produced.

D. Dissolve about 10 mg in 5 ml of *7 M hydrochloric acid* and allow to stand for about 20 minutes; a yellow colour develops.

Tests

pH (2.4.24). 8.0 to 10.5, determined in a 0.066 per cent w/v solution in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). –78.0° to –71.0°, determined in a 2.0 per cent w/v solution in *ethanol*. Measure the optical rotation at least 30 minutes after preparing the solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel H*.

Mobile phase. A mixture of 75 volumes of *methanol* and 45 volumes of a 5 per cent w/v solution of *ammonium acetate*.

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.2 per cent w/v solution of *erythromycin IPRS* in *methanol*.

Reference solution (b). A 0.01 per cent w/v solution of *erythromycin IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. Any secondary spot with an R_f value lower than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the principal spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 6.5 per cent, determined on 0.2 g using a 10 per cent w/v solution of *imidazole* in *anhydrous methanol* as the solvent.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10), using a solution prepared by dissolving about 25 mg, accurately weighed, in 10 ml of *methanol* and adding sufficient sterile *phosphate buffer pH 8.0* to produce 100.0 ml. Express the results as units per mg.

Storage. Store protected from light at a temperature not exceeding 30°.

Erythromycin Gastro-resistant Tablets

Erythromycin Tablets

Erythromycin Gastro-resistant Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of erythromycin, $C_{37}H_{67}NO_{13}$. They are made gastro-resistant by enteric-coating or by other means.

Usual strength. 250 mg.

Identification

A. Shake a quantity of the powdered tablets containing about 0.1 g of Erythromycin with 5 ml of *chloroform*, decolorise if necessary, with *decolorising charcoal*, filter and evaporate the filtrate to dryness. The residue after drying at a pressure not exceeding 0.7 kPa complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *erythromycin IPRS* or with the reference spectrum of erythromycin.

B. Dissolve a quantity of the powdered tablets containing about 3 mg of Erythromycin as completely as possible in 2 ml of *acetone* and add 2 ml of *hydrochloric acid*; an orange colour is produced which changes to red and then to deep purplish red. Add 2 ml of *chloroform* and shake; the chloroform layer becomes purple.

Tests

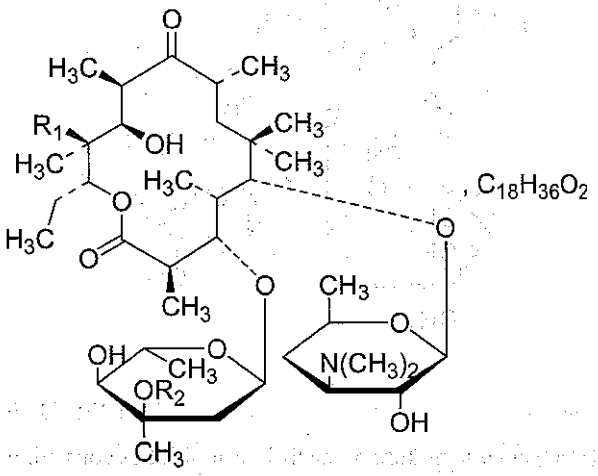
Other tests. Comply with the tests stated under Tablets.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.25 g of Erythromycin and triturate with 25 ml of *methanol* and add sufficient sterile *phosphate buffer pH 8.0* to produce 100.0 ml. Calculate the content of erythromycin in the tablets, taking each 1000 Units found to be equivalent to 1 mg of erythromycin.

Storage. Store protected from light at a temperature not exceeding 30°.

Erythromycin Stearate



$C_{37}H_{67}NO_{13},C_{18}H_{36}O_2$ Mol. Wt. 1018.4

Erythromycin Stearate is a mixture of the stearate of Erythromycin with an excess of stearic acid.

Erythromycin Stearate has a potency not less than 600 Units of erythromycin per mg, calculated on anhydrous basis.

Category. Antibacterial.

Description. Colourless or slightly yellow crystals or a white or slightly yellow, crystalline powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. The upper layer of a mixture of 45 volumes of *ethyl acetate*, 40 volumes of a 15 per cent w/v solution of

ammonium acetate, previously adjusted to pH 9.6 with *9 M ammonia*, and 20 volumes of *2-propanol*.

Test solution. Dissolve 0.28 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.2 per cent w/v solution of *erythromycin IPRS* in *methanol*.

Reference solution (b). A 0.1 per cent w/v solution of *stearic acid* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with a solution containing 0.02 per cent w/v of *2,7-dichlorofluorescein* and 0.01 per cent w/v of *rhodamine B* in *ethanol (95 per cent)*, allow the plate to stand for a few seconds in the vapour above a water-bath and examine under ultraviolet light at 365 nm. The chromatogram obtained with the test solution exhibits two spots, one of which corresponds in position to the principal spot in the chromatogram obtained with reference solution (a) and the other to the principal spot in the chromatogram obtained with reference solution (b). Spray the plate with *ethanolic anisaldehyde solution*, heat at 110° for 5 minutes and examine in daylight. The coloured spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a).

B. To about 5 mg add 5 ml of a 0.02 per cent w/v solution of *xanthydrol* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of *5 M acetic acid* and heat on a water-bath; a red colour is produced.

C. Dissolve about 10 mg in 5 ml of *7 M hydrochloric acid* and allow to stand for about 20 minutes; a yellow colour develops.

Tests

pH (2.4.24). 7.0 to 10.5, determined in a 1.0 per cent w/v suspension.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel H*.

Mobile phase. A mixture of 100 volumes of *methanol* and 60 volumes of a 15 per cent w/v solution of *ammonium acetate*.

Test solution. Dissolve 0.28 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.2 per cent w/v solution *erythromycin IPRS* in *methanol*.

Reference solution (b). A 0.01 per cent w/v solution of *erythromycin IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic anisaldehyde solution*, heat at 110° for 5 minutes and allow to cool. Any spot with an R_f value lower than that of the principal spot in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) and any spot with an R_f value higher than that of the principal spot is not more intense than the principal spot in the chromatogram obtained with reference solution (b).

Erythromycin stearate. Not less than 84.0 per cent of $C_{37}H_{67}NO_{13},C_{18}H_{36}O_2$, calculated on the anhydrous basis and determined by the following method. Weigh accurately about 0.5 g and dissolve in 30 ml of *chloroform*. If the solution is opalescent, filter and shake the residue with three quantities, each of 25 ml, of *chloroform*. Filter, if necessary, and wash the filter with *chloroform*. Evaporate the combined filtrate and washings on a water-bath to about 30 ml, add 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.1018 g of $C_{37}H_{67}NO_{13},C_{18}H_{36}O_2$.

Free stearic acid. Not more than 14.0 per cent of $C_{18}H_{36}O_2$, calculated on the anhydrous basis and determined by the following method. Weigh accurately about 0.4 g and dissolve in 50 ml of *methanol*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Calculate the volume of *0.1 M sodium hydroxide* required for each g of the substance and subtract the volume of *0.1 M perchloric acid* required for each g of the substance in the test for Erythromycin stearate.

1 ml of the difference is equivalent to 0.02845 g of $C_{18}H_{36}O_2$.

Erythromycin stearate and free stearic acid. 98.0 to 103.0 per cent, calculated by adding together the percentages of erythromycin stearate and free stearic acid determined as described above.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.3 g using a 10 per cent w/v solution of *imidazole* in *anhydrous methanol* as the solvent.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) using a solution prepared by dissolving about 50 mg accurately weighed in sufficient *methanol* to produce 100.0 ml. Express the results as units per mg.

Storage. Store protected from light at a temperature not exceeding 30°.

Erythromycin Stearate Tablets

Erythromycin Stearate Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of erythromycin, $C_{37}H_{67}NO_{13}$.

Usual strengths. The equivalent of 125 mg; 250 mg of erythromycin.

Identification

A. To a quantity of the powdered tablets containing 0.1 g of erythromycin add 10 ml of *water* and shake well. Decant the supernatant liquid and discard. Extract the residue by shaking with 10 ml of *methanol,* filter the extract and evaporate to dryness. The residue after drying at a pressure not exceeding 0.7 kPa complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *erythromycin stearate IPRS* or with the reference spectrum of erythromycin stearate.

B. Dissolve a quantity of the powdered tablets containing 3 mg of erythromycin as completely as possible in 2 ml of *acetone* and add 2 ml of *hydrochloric acid*; an orange colour is produced which changes to red and then to deep purplish red. Add 2 ml of *chloroform* and shake; the chloroform layer becomes purple.

C. Extract a quantity of the powdered tablets containing 50 mg of erythromycin with 10 ml of *chloroform*, filter and evaporate to dryness. Heat 0.1 g of the residue gently with 5 ml of *2 M hydrochloric acid* and 10 ml of *water* until the solution boils; oily globules rise to the surface. Cool, remove the fatty layer, heat it with 3 ml of *0.1 M sodium hydroxide* and allow to cool; the solution sets to a gel. Add 10 ml of hot *water* and shake; the solution froths. To 1 ml add a 10 per cent w/v solution of *calcium chloride;* a granular precipitate is produced which is insoluble in *hydrochloric acid.*

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 2.72 per cent w/v solution of *sodium acetate,* adjusted to pH 5.0 with *glacial acetic acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of medium and filter. To 5 ml of the filtrate, add 40 ml of *glacial acetic acid* and 10 ml of a 0.5 per cent w/v solution of *4- dimethylaminobenzaldehyde* in *glacial acetic acid* and dilute to 100 ml with a mixture of 35 volumes of *glacial acetic acid* and 70 volumes of *hydrochloric acid.* Allow to stand for 15 minutes and measure the absorbance of the resulting solution at the maximum at 485 nm (2.4.7). Calculate the content of $C_{37}H_{67}NO_{13}$ in the medium from the absorbance obtained from a solution of known concentration of *erythromycin stearate IPRS* prepared in the same manner.

Q. Not less than 70 per cent of the stated amount of $C_{37}H_{67}NO_{13}$.

Other tests. Comply with the tests stated under Tablets.

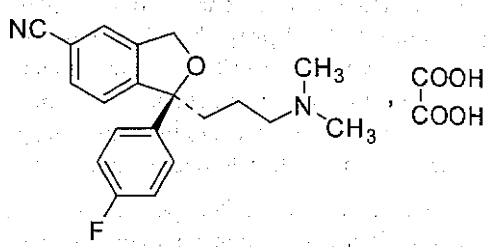
Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of erythromycin and dissolve as completely as possible in sufficient *methanol* to produce 100.0 ml. Calculate the content of erythromycin in the tablets, taking each 1000 Units found to be equivalent to 1 mg of erythromycin.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of erythromycin.

Escitalopram Oxalate



$C_{20}H_{21}FN_2O,C_2H_2O_4$ Mol. Wt. 414.4

Escitalopram Oxalate is (*S*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile oxalate.

Escitalopram Oxalate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{21}FN_2O.C_2H_2O_4$, calculated on the anhydrous basis.

Category. Antidepressant.

Description. A white to slightly yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *escitalopram oxalate IPRS* or with the reference spectrum of escitalopram oxalate.

Tests

Specific optical rotation (2.4.22). +10.0° to +13.0°, determined in 1.0 per cent w/v solution in the *methanol*.

Enantiomeric Purity. Not more than 2.0 per cent of R- isomer.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. Dissolve 25 mg of *citalopram hydrombromide IPRS* in 2.5 ml of *methanol* and dilute to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with OD-H (5 µm) (Such as Chirelcel),
- column temperature: 30°,
- mobile phase: a mixture of 90 volumes of *n-hexane*, 10 volumes of *ethanol* and 0.4 volume of *trifluoroacetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resoluiton between the peaks due to *S*-isomer and *R*-isomer is not less than 1.5. The relative retention time with reference to S- isomer for R- isomer is about 1.2.

Inject the reference solution and the test solution.

Calculate the content of R- isomer.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Prepare the solutions immediately before use.

Test solution. Dissolve about 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). A 0.0005 per cent w/v solution of *escitalopram oxalate IPRS* in the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *oxalic acid* in the mobile phase.

Use chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the sum of the area of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore the peak due to oxalic acid.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *escitalopram oxalate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Waters-Xterra),
- column temperature: 40°,
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 4.45 g of *disodium hydrogen phosphate dihydrate* in 1000 ml of *0.1 per cent triethylamine*, adjusted to pH 8.5 with *orthophosphoric acid* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}FN_2O.C_2H_2O_4$.

Storage Store protected from moisture.

Escitalopram Tablets

Escitalopram Oxalate Tablets

Escitalopram Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of escitalopram, $C_{20}H_{21}FN_2O$.

Usual strengths. 5 mg; 10 mg; 20 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), as described under Assay using the following solutions.

Test solution. The filtrate obtained as given above, diluted if necessary with the dissolution medium.

Reference solution. A solution of *escitalopram oxalate IPRS* containing about 0.00055 per cent w/v of escitalopram in the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{20}H_{21}FN_2O$.

Uniformity of content. Complies with the test stated under tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following solution as the test solution.

Test solution. Disperse 1 tablet in the mobile phase, sonicate and dilute, if necessary to obtain a solution containing 0.001 per cent w/v of escitalopram in the mobile phase.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 25 mg of Escitalopram with 30 ml of mobile phase, sonicate to dissolve and dilute to 50.0 ml with the mobile phase. Further dilute to obtain a 0.001 per cent w/v solution of escitalopram.

Reference solution. A solution of *escitalopram oxalate IPRS* containing about 0.001 per cent w/v of escitalopram in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of buffer solution prepared by dissolving 3.7 g of *disodium hydrogen phosphate dihydrate* in 1000 ml of *water*, adjusted to pH 7.0 with *orthophosphoric acid*, 27.5 volumes of *methanol* and 27.5 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}FN_2O$ in the tablets.

Labelling. The label states the strength in terms of the equivalent amount of Escitalopram.

Escitalopram Oxalate and Clonazepam Tablets

Escitalopram and Clonazepam Tablets.

Escitalopram Oxalate and Clonazepam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of escitalopram, $C_{20}H_{21}FN_2O$ and clonazepam, $C_{15}H_{10}ClN_3O_3$.

Usual strength. Escitalopram Oxalate equivalent to Escitalopram,10 mg and Clonazepam, 0.25 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with the reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *Water,*
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter, rejecting first few ml of the filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above, diluted suitably if necessary, with the dissolution medium.

Reference solution. Dissolve an accurately weighed quantity of *escitalopram oxalate IPRS* and *clonazepam IPRS* in 5.0 ml *methanol* and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Use chromatographic system as described under Assay with the following modification.

- spectrophotometer set at 210 nm,
- injection volume: 100 µl.

Inject the reference solution and the test solution.

Calculate the contents of $C_{20}H_{21}FN_2O$ and $C_{15}H_{10}ClN_3O_3$.

Q. Not less than 70.0 per cent of the stated amounts of $C_{20}H_{21}FN_2O$ and $C_{15}H_{10}ClN_3O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Use chromatographic system, solvent mixture and test solution as described under Assay.

Inject the test solution. The area of any secondary peak is not more than 1.0 per cent and the sum of areas of all the secondary

peaks is not more than 2.0 per cent, calculated by area normalisation.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Disperse one tablet with 20 ml of *solvent mixture* for 30 minutes, add sufficient *solvent mixture* to produce 25.0 ml, mix and filter.

Use chromatographic system, reference solution (b) and solvent mixture as described under Assay.

Inject reference solution (b) and the test solution.

Calculate the contents of $C_{20}H_{21}FN_2O$ and $C_{15}H_{10}ClN_3O_3$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 400 volumes of *water*, 300 volumes of *methanol* and 300 volumes of *acetonitrile.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 40 mg of Escitalopram with 50 ml of *solvent mixture*, ultrasound for 20 minutes, add sufficient *solvent mixture* to produce 100.0 ml, mix and filter.

Reference solution (a). Dissolve 10 mg of *clonazepam IPRS* in 50.0 ml of solvent mixture, ultrasound to dissolve and add sufficient solvent mixture to produce 100.0 ml.

Reference solution (b). Dissolve 25.7 mg of *escitalopram oxalate IPRS* in 40.0 ml of solvent mixture, add 5.0 ml reference solution (a) ultrasound and add sufficient solvent mixture to produce 50.0 ml.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane chemically bonded to porous silica (5 µm) (Such as Hypersil BDS),
- mobile phase: a mixture of 550 volumes of a buffer solution prepared by dissolving 6.8 g of *Potassium dihydrogen orthopsosphate* and 8.7 g of *dipotassium hydrogen orthophosphate* in 1000 ml of *water*, add 3 ml of *triethylamine* and adjusted to pH 6 with *orthophosphoric acid*, 315 volumes of *methanol*, and 135 volumes of *acetonitrile,*
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The retention time of peaks due to escitalopram is about 10 minutes and clonazepam is about 14 minutes.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 5000 theoretical plates for escitalopram peak, tailing factor is not more than 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

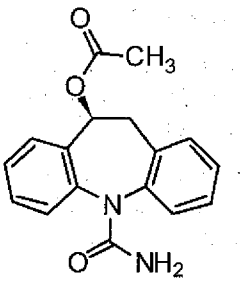
Inject reference solution (b) and the test solution.

Calculate the contents of $C_{20}H_{21}FN_2O$ and $C_{15}H_{10}ClN_3O_3$ in the tablets.

Storage. Store protected from moisture, at the temperature not exceed 30°.

Labelling. The label states the strength in terms of the equivalent amount of escitalopram and the quantity of clonazepam.

Eslicarbazepine Acetate



$C_{17}H_{16}N_2O_3$ Mol Wt. 296.3

Eslicarbazepine Acetate is (*S*)-10-Acetoxy- 10,11-dihydro-5*H*-dibenz[b,f]azepine- 5-carboxamide.

Eslicarbazepine Acetate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{16}N_2O_3$, calculated on the anhydrous basis.

Category. Antiepileptic

Description. A white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *eslicarbazepine acetate IPRS* or with the reference spectrum of eslicarbazepine acetate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *water* and 50 volumes of *methanol.*

Test solution. Dissolve about 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A 0.0005 per cent w/v solution of *eslicarbazepine acetate IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* , *acid* and 30 volumes of a mixture of 60 volumes of *acetonitrile* and 40 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.3 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *water* and 50 volumes of *methanol*.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *eslicarbazepine acetate IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid*, *acid* and 30volumes of a mixture of 60 volumes of *acetonitrile* and 40 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0.and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{16}N_2O_3$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Eslicarbazepine Tablets

Eslicarbazepine Acetate Tablets

Eslicarbazepine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of eslicarbazepine acetate, $C_{17}H_{16}N_2O_3$.

Usual strengths. 200 mg; 400 mg; 600 mg; 800 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 0.5 per cent w/v solution of *sodium lauryl sulphate* in *water*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 44 mg of *eslicarbazepine acetate IPRS* in 10 ml of *methanol* with the aid of ultrasound and dilute to 100.0 ml with the dissolution medium. Dilute 5.0 ml of the solution to 50.0 ml with the dissolution medium.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 1.36g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid*, 50 volumes of a mixture of 60 volumes of *acetonitrile* and 40 volumes of *methanol*,

- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{16}N_2O_3$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{17}H_{16}N_2O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of buffer solution and *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 100 mg of Eslicarbazepine Acetate in 120 ml of the solvent mixture with the aid of ultrasound for 25 minutes with intermittent shaking and dilute to 200.0 ml with the solvent mixture and filter.

Reference solution. A 0.00025 per cent w/v solution of *eslicarbazepine acetate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 30 volumes of a mixture of 60 volumes of *acetonitrile* and 40 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of buffer solution and *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 100 mg of Eslicarbazepine Acetate in 120 ml of the solvent mixture with the aid of ultrasound for 25 minutes with intermittent shaking and dilute to 200.0 ml with the solvent mixture, filter. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *eslicarbazepine acetate IPRS* in the solvent mixture.

Use chromatographic system as described under Related substances with the following modification.

- a mixture of 50 volumes of buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid*, 50 volumes of a mixture of 60 volumes of *acetonitrile* and 40 volumes of *methanol*,

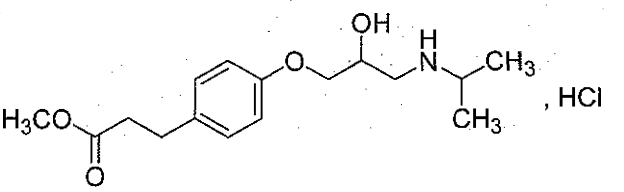
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{16}N_2O_3$ in tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Esmolol Hydrochloride



$C_{16}H_{25}NO_4,HCl$ Mol. Wt. 331.8

Esmolol Hydrochloride is Methyl (*RS*)-3-[4-(2-hydroxy-3-isopropylaminopropoxy)phenyl]propanoate hydrochloride.

Esmolol Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{25}NO_4,HCl$, calculated on the anhydrous basis.

Category. Antihypertensive.

Description. A white to off white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *esmolol hydrochloride IPRS* or with the reference spectrum of esmolol hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 3.0 to 5.0, determined in 25.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in *water* and dilute to 100.0 ml with *water*.

Reference solution (a). A 0.001 per cent w/v solution of *esmolol hydrochloride IPRS* in *water*.

Reference solution (b). A 0.1 per cent w/v solution of *esmolol hydrochloride IPRS* in *1 M hydrochloric acid*. Allow the contents to stand for at least 30 minutes. *(NOTE— This results in the partial degradation of the esmolol resulting in the production of esmolol free acid)*. Dilute 1.0 ml of the solution to 5.0 ml with *water*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: A. *methanol*,
  B. a mixture of 65 volumes of buffer solution prepared by dissolving 3.0 g of *potassium dihydrogen phosphate* in 650 ml of *water*, 15 volumes of *acetonitrile* and 20 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent w/v)
0	0	100
20	0	100
25	25	75
35	25	75
36	0	100
40	0	100

Name	Relative retention time
Esmolol free acid[1]	0.43
Esmolol dimer[2]	6.5
Esmolol isopropylamide analog[3] (if present)	0.65
N-ethyl esmolol[4]	0.84
Esmolol	1.0

[1]3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl}propanoic acid,

[2]methyl 3-{4-[2-hydroxy-3-(3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl}-*N*-isopropylpropanamido)propoxy]phenyl}propanoate,

[3]3-{4-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl}-*N*-isopropylpropionamide,

[4]methyl 3-{4-[3-(ethylamino)-2-hydroxypropoxy]phenyl}propionate.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to esmolol free acid and esmolol is not less than 4.0 and the tailing factor is not more than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to esmolol free acid is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent), the area of any peak corresponding to esmolol dimer is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any peak corresponding to esmolol isopropylamide analog is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent), the area of any peak corresponding to N-ethyl esmolol is not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g using Method 1.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in *water* and dilute to 100.0 ml with *water*.

Reference solution (a). A 0.02 per cent w/v solution of *esmolol hydrochloride IPRS* in *water*.

Reference solution (b). A 0.1 per cent w/v solution of *esmolol hydrochloride IPRS* in *1 M hydrochloric acid*. Allow to stand for 30 minutes. *(NOTE— This results in the partial degradation of the esmolol resulting in the production of esmolol free acid)*. Dilute 1.0 ml of the solution to 5.0 ml with *water*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 65 volumes of buffer solution prepared by dissolving 3.0 g of *potassium dihydrogen phosphate* in 650 ml of *water*, 15 volumes of *acetonitrile* and 20 volumes of *methanol*.
- flow rate: 2 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 20 µl.

The relative retention time with reference to esmolol for esmolol free acid is about 0.41.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to esmolol free acid and esmolol is not less than 4.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{25}NO_4,HCl$.

Storage. Store at a temperature not exceeding 30°.

Esmolol Injection

Esmolol Hydrochloride Injection

Esmolol Hydrochloride Injection is a sterile solution of Esmolol Hydrochloride in Water for Injections.

Esmolol Hydrochloride Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of esmolol hydrochloride, $C_{16}H_{25}NO_4,HCl$.

Usual Strength. 10 mg per ml.

Description. A clear, almost colourless solution.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 5.5.

Bacterial Endotoxins (2.2.3). Not more than 3.5 Endotoxin Units per mg of Esmolol Hydrochloride.

Other tests. Comply with the test stated under Parenteral Preparations (Injections).

Assay. Determined by liquid Chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing 50 mg of Esmolol Hydrochloride to 100.0 ml with the mobile phase.

Reference solution. A 0.05 per cent w/v solution of *esmolol hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 3.0 g of *monobasic potassium dihydrogen phosphate* in 650 ml of *water*, 20 volumes of *methanol* and 15 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 20 µl.

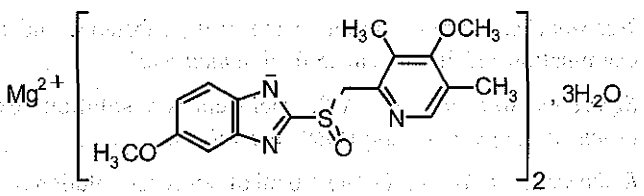
Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{25}NO_4,HCl$ in the injection.

Storage. Store protected from light at a temperature below 25°.

Esomeprazole Magnesium Trihydrate



$(C_{17}H_{18}N_3O_3S)_2,Mg.3H_2O$ Mol. Wt. 767.2

Esomeprazole Magnesium Trihydrate is 5-methoxy-2-{(*S*)-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl}-1*H*-benzimidazole magnesium trihydrate.

Esomeprazole Magnesium Trihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{17}H_{18}N_3O_3S)_2,Mg$, calculated on the anhydrous basis.

Category. Antiulcer.

Description. A white to slightly coloured powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *esomeprazole magnesium IPRS* or with the reference spectrum esomeprazole magnesium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Magnesium. 3.0 per cent to 3.7 per cent, calculated on the anhydrous basis and determined in the following manner.

Weigh 1 g of the substance under examination and ignite in a silica crucible until no fumes are evolved. Cool and moisten the residue with 0.5 ml of *sulphuric acid*. Evaporate the acid until no white fumes are noticed. Heat the residue at 800° for 2 hours. Cool the residue to room temperature and dissolve in 5 ml of *dilute hydrochloric acid* with the aid of ultrasound for 5 minutes. Transfer the residue to a flask, wash with about 15 ml of *water* and transfer the washing to the flask. Repeat the washing four to five times and add the washings to the extract in the flask. Add 5 ml of a mixture of 180 g of *ammonium chloride* and 750 ml of *strong ammonia solution* and sufficient *water* to produce 1000 ml and 200 mg of *eriochrome black T mixture*. Titrate with *0.05 M disodium edetate*.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0012155 g of magnesium.

Specific optical rotation (2.4.22). – 155° to – 135°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse about 25 mg of the substance under examination and dissolve in 25.0 ml of *methanol*.

Reference solution (a). A 0.1 per cent w/v solution of *esomeprazole magnesium IPRS* in *methanol*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of a buffer solution prepared by dissolving 1.2 g of *ammonium dihydrogen orthophosphate* in 1000 ml of *water* adding 5 ml of *triethylamine* and adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). 6.0 per cent to 11.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *0.01 M sodium borate* and 25 volumes of *acetonitrile*.

Test solution. Disperse about 20 mg of the substance under examination in 10 ml of *methanol* and dilute to 100.0 ml with the solvent mixture.

Reference solution. Disperse about 20 mg of the *esomeprazole magnesium IPRS* in 10 ml of *methanol* and dilute to 100.0 ml with the solvent mixture.

Use the chromatographic system described under the test for Related substances.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Storage. Store protected from moisture.

Esomeprazole Gastro-resistant Capsules

Esomeprazole Capsules; Esomeprazole Magnesium Gastro-resistant Capsules; Esomeprazole Magnesium Capsules

Esomeprazole Gastro-resistant Capsules contains not less than 90.0 per cent and not more than 110.0 per cent of esomeprazole, $C_{17}H_{19}N_3O_3S$.

Usual strengths. 20 mg; 40 mg.

Identification

Enantiomeric purity (see Tests).

Tests

Dissolution (2.5.2).

A. Apparatus No. 2 (Paddle),

Medium: 900 ml of *0.1 M hydrochloric acid.*
Speed and time. 100 rpm and 2 hours.

Tap the granules from a capsule slightly with a glass rod to make them settle to the bottom. Rotate the paddle at 100 rpm for 2 hours, drain the solution slowly without losing any granules. Transfer them quantitatively to a 100-ml volumetric flask, add 20 ml of *0.1 M sodium hydroxide* and mix with the aid of ultrasound. Dilute to volume with *0.1 M sodium hydroxide,* centrifuge about 15 ml for 5 minutes and dilute 5.0 ml of the clear supernatant liquid to 50.0 ml with the mobile phase. Using the resulting solution as the test solution, carryout the determination as described under Assay. Calculate the content of $C_{17}H_{19}N_3O_3S$ in the supernatant liquid. Calculate the percentage of esomeprazole released in the acid medium by subtracting the content of $C_{17}H_{19}N_3O_3S$ in the test solution from the total content of esomeprazole determined in the Assay.

Complies with the acceptance criteria given under acid stage.

B. Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 6.8,*
Speed and time. 100 rpm and 45 minutes.

Tap the granules from a capsule slightly with a glass rod to make them settle to the bottom. Rotate the paddle at 100 rpm for 45 minutes and filter the solution. Immediately transfer 5.0 ml of the solution to a test tube containing 1.0 ml of *0.1 M sodium hydroxide.* Prepare the standard solution having a known concentration similar to the expected concentration and in similar manner to the test solution. Calculate the content of $C_{17}H_{19}N_3O_3S$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{17}H_{19}N_3O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solution and protected from light.

Solvent mixture. Dissolve 5.24 g of *trisodium orthophosphate* in *water*, add 110.0 ml of *0.5 M disodium hydrogen orthophosphate* and make up the volume to 1000.0 ml with *water*, adjusted to pH 11.0 with *0.1 M sodium hydroxide solution.*

Buffer solution. Mix 5.2 ml of *1.0 M disodium hydrogen orthophosphate* buffer and 63.0 ml of *0.5 M sodium dihydrogen orthophosphate* buffer diluted to 1000.0 ml with *water*, adjusted to pH 7.6 with *0.1 M sodium hydroxide solution.*

Test solution. Disperse a quantity of mixed contents of capsules containing 80 mg of esomeprazole in 20.0 ml *methanol*, add 40.0 ml solvent mixture and mix with the aid of ultrasound for 5 minutes and dilute to 200.0 ml with the *water.*

Reference solution. Dissolve 10 mg each of *omeprazole IPRS* and *omeprazole sulphone IPRS* (omeprazole impurity A) in 100.0 ml *methanol.* Dilute 1.0 ml of the solution to 100.0 ml in a mixture of 1 volume of solvent mixture and 4 volume of *water.*

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porus silica (3 µm),
- mobile phase: A. a mixture of 100 ml of *acetonitrile* and 100 ml of buffer solution and diluted to 1000 ml with *water*,
  B. a mixture of 800 ml of *acetonitrile* and 10 ml of buffer solution and diluted to 1000 ml with *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
10	80	20
30	0	100
31	100	0
45	100	0

The relative retention time with reference to omeprazole for omeprazole sulphone is 0.93.

Inject the reference solution. The test is not valid unless resolution between the peaks due to omeprazole and omeprazole impurity A is not less than 2.5.

Inject the test solution. In the chromatogram obtained with the test solution the area of peak corresponding to omeprazole impurity A is not more than 0.5 per cent, the area of any other secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization.

Enantiomeric purity. Determined by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 5.24 g of *trisodium phosphate* in *water*, add 110 ml of *0.5 M disodium hydrogen phosphate* and make up the volume to 1000 ml with *water* and adjusted to pH 11.0.

Test solution. Shake a quantity of the mixed contents of 20 capsules containing 20 mg of esomeprazole in 120 ml solvent mixture, add 40 ml *ethanol* and mix with the aid of ultrasound for 5 minutes and dilute to 200 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml in *water.*

Reference solution. Dissolve 20 mg of *omeprazole IPRS* in 120 ml solvent mixture, add 40 ml *ethanol* and mix with the aid

of ultrasound for 5 minutes and dilute to 200 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml in *water*.

Chromatographic system

- a stainless steel column 10 cm x 4.0 mm, packed with immobilized alpha 1 acid glycoprotein on special silica particles (5 µm),
- mobile phase: a mixture of 150 ml of *acetonitrile* and 85 ml of buffer solution prepared by dissolving 26.6 g of *disodium hydrogen orthophosphate* and 55.2 g *sodium dihydrogen orthophosphate* in 1000 ml *water*, adjusted to pH 6.0 and finally diluted to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume: 20 µl.

The elution order of enantiomers is R-enantiomer and then S-enantiomer which is esomeprazole peak.

Inject the reference solution and the test solution. The test is not valid unless the resolution between the peaks corresponding to esomeprazole and enantiomer R is not less than 1.0. The ratio of the retention time of esomeprazole peak in the reference solution and the test solution should be between 0.98 and 1.02.

Other tests. Comply with the tests stated under Capsules.

Assay. Determined by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 5.24 g of *trisodium phosphate* in *water*, add 110 ml of *0.5 M di-sodium hydrogen phosphate* and make up the volume to 1000 ml with *water* and adjusted to pH 11.0 with *0.1 M sodium hydroxide solution*.

Test solution. Weigh and mix the contents of 20 capsules. Disperse a quantity of the mixed content containing about 20 mg of esomeprazole with 60 ml of the solvent mixture, shake for 20 minutes to dissolve the pellets. Add 20 ml of *methanol* and mix with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Dilute 2.0 ml of the solution to 10.0 ml using *water*, filter. Store the solution protected from light.

Reference solution. Dissolve 10 mg *omeprazole IPRS* in 10 ml *methanol*, add 40 ml solvent mixture and further dilute with *water* to obtain a concentration of 0.004 per cent of omeprazole.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porus silica (5 µm),
- mobile phase: a mixture of 350 ml of *acetonitrile*, 500 ml of buffer solution prepared by mixing 10.5 ml *1.0 M sodium dihydrogen orthophosphate* and 60.0 ml *0.5 M disodium hydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.3 and finally diluted to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_3O_3S$ in the capsules.

Storage. Store protected from moisture at room temperature.

Labelling. The label state the strength in terms of equivalent amount of esomeprazole.

Esomeprazole Gastro-resistant Tablets

Esomeprazole Tablets; Esomeprazole Magnesium Gastro-resistant Tablets; Esomeprazole Magnesium Tablets

Esomeprazole Gastro-resistant Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of esomeprazole, $C_{17}H_{19}N_3O_3S$. They are made gastro-resistant by enteric-coating or by other means.

Usual strengths. 20 mg; 30 mg; 40 mg.

1 mg of Esomeprazole magnesium trihydrate $(C_{17}H_{18}N_3O_3S)_2.Mg.3H_2O$ is equivalent to 900.5 µg of Esomeprazole $(C_{17}H_{19}N_3O_3S)_2$.

Identification

In the Assay the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

NOTE — Perform all the tests in subdued light using low actinic glassware.

Dissolution (2.5.2).

A. Apparatus No. 2 (Paddle),
Medium. 1000 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 2 hours.

Determine by liquid chromatography (2.4.14).

Test solution. Withdraw the medium completely and disperse the intact tablet in suitable amount of *methanol* to obtain a solution containing 0.02 per cent w/v of esomeprazole. Centrifuge at about 4000 rpm for 10 minutes. Dilute 5.0 ml of the solution to 25.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *esomeprazole magnesium IPRS* in *methanol*. Dilute 5.0 ml of the solution to 25.0 ml with the mobile phase.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_3O_3S$ released in the acid medium by subtracting the content of $C_{17}H_{19}N_3O_3S$ in the test solution from the total content of esomeprazole $C_{17}H_{19}N_3O_3S$ determined in the Assay.

Complies with the acceptance criteria given under acid stage.

B. Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 75 rpm and 60 minutes.

Transfer another 6 tablets and run the apparatus for 2 hours in *0.1 M hydrochloric acid*. Decant the medium without losing the tablets, add phosphate buffer pH 6.8 and run the apparatus for 60 minutes. Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.022 per cent w/v solution of *esomeprazole magnesium IPRS* in *methanol*. Dilute a suitable volume of the solution with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_3O_3S$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{17}H_{19}N_3O_3S$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.24 g of esomeprazole, add about 150 ml of *methanol* and mix with the aid of ultrasound for 15 minutes. Mix and add sufficient *methanol* to produce 250.0 ml. Mix with the aid of ultrasound for a further 5 minutes and centrifuge. Dilute 5.0 ml of the clear supernatant liquid to 25.0 ml with the mobile phase.

Reference solution. Weigh 0.055 g of *esomeprazole magnesium IPRS*, dissolve in 50 ml of *methanol*, add sufficient mobile phase to produce 250.0 ml and mix well.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *methanol* and a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* and about 1 g of *sodium hydroxide* in 1000 ml of *water* and adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 50 µl.

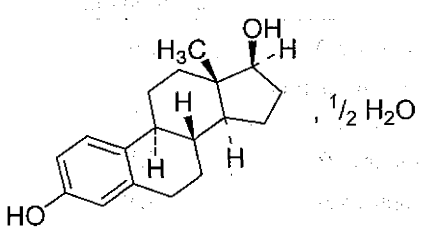
Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of the replicate injections is not more than 2.0 per cent.

Calculate the content of $C_{17}H_{19}N_3O_3S$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of esomeprazole.

Estradiol Hemihydrate



$C_{18}H_{24}O_2, ½H_2O$ Mol. Wt. 281.4

Estradiol Hemihydrate is Estra-1,3,5(10)-triene-3,17β-diol hemihydrate.

Estradiol Hemihydrate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{18}H_{24}O_2, ½ H_2O$, calculated on the anhydrous basis.

Category. Estrogen.

Description. A white or almost white, crystalline powder or colourless crystals.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *estradiol hemihydrate IPRS* or with the reference spectrum of estradiol hemihydrate.

Tests

Specific optical rotation (2.4.22). + 76.0° to + 83.0°, determined in 1.0 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of *acetonitrile* and dilute to 25.0 ml with the *methanol*.

Reference solution (a). A 0.0002 per cent w/v solution of *estradiol hemihydrate IPRS* in *methanol*.

Reference solution (b). A mixture of equal volumes of 0.1 per cent w/v solution, each of, *estradiol hemihydrate IPRS* and *2, 3-dichloro-5,6-dicyanobenzoquinone* in *methanol*. Allow to stand for 30 minutes before injection, to generate impurity D.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile*, 55 volumes of *water* and 5 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Estradiol impurity D[1]	0.9	0.4
Estradiol (Retention time is about 13 minutes)	1.0	—
Estradiol impurity B[2]	1.1	—
Estradiol impurity A[3]	1.4	—
Estradiol impurity C[4]	1.9	—

[1]estra-1,3,5(10), 9(11)-tetraene-3,17β-diol,

[2]estra-1,3,5(10)-triene-3,17α-diol (17α-estradiol),

[3]3-hydroxyestra-1,3,5(10)-trien-17-one (estrone),

[4]4-methylestra-1,3,5(10)-triene-3,17β-diol.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to estradiol and impurity D is not less than 2.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to impurity A, B, C and D, each of, is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (2.3.43). 2.9 per cent to 3.5 per cent, determined on 0.5 g.

Assay. Dissolve 20.0 mg of substance under examination in *ethanol (95 per cent)* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *0.1M sodium hydroxide*. Allow to cool to room temperature and measure the absorbance of the resulting solution at the maximum at about 238 nm (2.4.7). Calculate the content of $C_{18}H_{24}O_2$ taking 335 as the specific absorbance at 238 nm.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Estradiol and Norethisterone Tablets

Estradiol and Norethisterone Acetate Tablets

Estradiol and Norethisterone Tablets contain Estradiol Hemihydrate and Norethisterone Acetate.

Estradiol and Norethisterone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of estradiol, $C_{18}H_{24}O_2$ and norethisterone acetate, $C_{22}H_{28}O_3$.

Usual Strength. Estradiol 2 mg and norethisterone 1 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *acetone* and 90 volumes of *dichloromethane*.

Test solution. Add 0.2 ml of *water* to two tablets and shake to disperse. Add sufficient *ethanol (95 per cent)* to produce a solution containing 0.035 per cent w/v of Norethisterone Acetate, centrifuge and use the clear supernatant liquid.

Reference solution. A suitable concentration of *estradiol hemihydrate IPRS* and *norethisterone acetate IPRS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Spray with *ethanolic sulphuric acid (5 per cent)* and heat the plate at 105° for 15 minutes. The chromatogram obtained with test solution shows two clearly separated spots with R_f values corresponding to those observed in the chromatogram obtained with the reference solution.

B. In the test for Uniformity of content, the chromatogram obtained with the test solution shows two peaks with the same retention times as the peaks due to estradiol and norethisterone acetate in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 500 ml of a 0.3 per cent w/v solution of *sodium lauryl sulphate* in *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, discarding the first 5 ml of filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate and, if necessary, dilute with the dissolution medium.

Reference solution (a). Dissolve a sufficient quantity of *estradiol hemihydrate IPRS* and *norethisterone acetate IPRS* in *methanol (80 per cent)* and dilute with dissolution medium; the concentration of the final solution should be the same as that expected for test solution.

Reference solution (b). A solution containing 0.0017 per cent w/v of *estradiol hemihydrate IPRS*, 0.00084 per cent w/v of *norethisterone acetate IPRS*, 0.00066 per cent w/v of *estrone IPRS* and 0.00034 per cent w/v of *norethisterone IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 2),
- mobile phase: a mixture of 45 volumes of *water* and 55 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 200 µl.

Inject reference solution (b). The test is not valid unless the resolution between each pair of peaks (estradiol and norethisterone, estrone and norethisterone acetate) is not less than 1.0.

Inject reference solution (a) and the test solution.

Calculate the total content of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ in the medium.

Estrone and norethisterone. Determine by liquid chromatography (2.4.14).

Test solution (a). Weigh and powder 20 tablets. Add 20 ml of the mobile phase to a quantity of the powdered tablets containing 5 mg of estradiol, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 ml. Centrifuge and use the clear supernatant liquid.

Test solution (b). Add 20 ml of the mobile phase to a quantity of the powdered tablets containing 2.5 mg of Norethisterone Acetate, mix with the aid of ultrasound and add sufficient mobile phase to produce 25 ml. Centrifuge and use the clear supernatant liquid.

Reference solution (a). A 0.0001 per cent w/v solution of *estrone IPRS* in the mobile phase.

Reference solution (b). A 0.00005 per cent w/v solution of *norethisterone IPRS* in the mobile phase.

Reference solution (c). A solution containing 0.0017 per cent w/v of *estradiol hemihydrate IPRS*, 0.00084 per cent w/v of *norethisterone acetate IPRS*, 0.00066 per cent w/v of *estrone IPRS* and 0.00034 per cent w/v of *norethisterone IPRS* in the mobile phase.

Use the chromatographic condition as described under Dissolution.

Inject 20 µl of reference solution (c). The test is not valid unless the resolution between each pair of peaks (estradiol and norethisterone, estrone and norethisterone acetate) is not less than 1.0.

Inject 20 µl of reference solution (a), (b), test solution (a) and (b). In the chromatogram obtained with test solution (a), the area of any peak corresponding to estrone is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). In the chromatogram obtained with test solution (b) the area of any peak corresponding to norethisterone is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Powder one tablet and add 20 ml of the mobile phase, mix with the aid of ultrasound, cool, add sufficient of the mobile phase to produce 25 ml and centrifuge. Dilute the supernatant liquid if necessary, with the mobile phase to produce a solution containing 0.002 per cent w/v of estradiol.

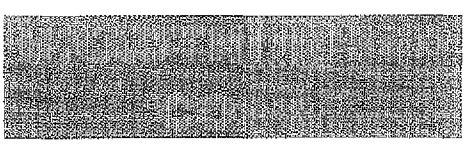
Reference solution (a). Dissolve sufficient quantities of *estradiol hemihydrate IPRS* and *norethisterone acetate IPRS* in the mobile phase and dilute an aliquot with the mobile phase; the concentrations in the final solution are the same as those expected for test solution.

Reference solution (b). A solution containing 0.0017 per cent w/v of *estradiol hemihydrate IPRS*, 0.00084 per cent w/v of *norethisterone acetate IPRS*, 0.00066 per cent w/v of *estrone IPRS* and 0.00034 per cent w/v of *norethisterone IPRS* in the mobile phase.

Use chromatographic system as described under Dissolution.

Inject 20 µl of reference solution (a), (b) and the test solution. The test is not valid unless the resolution between each pair of peaks (estradiol and norethisterone, estrone and norethisterone acetate) is not less than 1.0.

Calculate the contents of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ in the tablet.



Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Uniformity of content using the following test solution.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 10 mg of estradiol, add 20 ml of the mobile phase, mix with the aid of ultrasound, cool, add sufficient of the mobile phase to produce 25 ml and centrifuge. Dilute the supernatant liquid if necessary, with the mobile phase to produce a solution containing 0.002 per cent w/v of estradiol.

Inject 20 µl of reference solution (a) and the test solution.

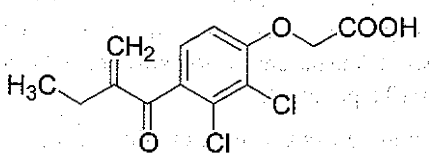
Calculate the contents of $C_{18}H_{24}O_2$ and $C_{22}H_{28}O_3$ in the tablets.

Storage. Store protected from light.

Labelling. The label states the quantity of estradiol hemihydrate in terms of the equivalent amount of estradiol.

Ethacrynic Acid

Etacrynic Acid



$C_{13}H_{12}Cl_2O_4$ Mol. Wt. 303.1

Ethacrynic Acid is 2-[2,3-dichloro-4-(2-ethylacryloyl) phenoxy]acetic acid

Ethacrynic Acid contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{13}H_{12}Cl_2O_4$, calculated on the dried basis.

Category. Diuretic.

Description. A white or almost white, crystalline powder.

CAUTION — As Ethacrynic Acid irritates the skin, eyes and the mucous membranes it should be handled with care.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethacrynic acid IPRS* or with the reference spectrum of ethacrynic acid.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in a mixture of 99 volumes of *methanol* and 1 volume of *1 M hydrochloric acid* shows a well defined absorption maximum at 270 nm and a shoulder at 285 nm; absorbance at 270 nm, 0.55 to 0.60.

C. To 25 mg add 2 ml of *1 M sodium hydroxide* and heat in a water-bath for 5 minutes, cool, add 0.25 ml of *sulphuric acid (50 per cent v/v)* and 0.5 ml of a 10 per cent w/v solution of *chromotropic acid sodium salt* and add cautiously 2 ml of *sulphuric acid*; a deep violet colour is produced.

D. On 20 mg determine by the oxygen-flask method (2.3.34), using 5 ml of *dilute sodium hydroxide solution* as the absorbing liquid. When the process is complete, acidify with *dilute sulphuric acid* and boil gently for 2 minutes; the solution gives the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *chloroform*, 50 volumes of *ethyl acetate* and 20 volumes of *glacial acetic acid*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent)*.

Reference solution (a). A 0.03 per cent w/v solution of the substance under examination in *ethanol (95 per cent)*.

Reference solution (b). A 0.01 per cent w/v solution of the substance under examination in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 2.0 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure of 0.1 to 0.5 kPa.

Assay. Weigh 0.25 g, dissolve in 100 ml of *methanol* and add 5 ml of *water*. Titrate with *0.1 M sodium hydroxide solution*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03031 g of $C_{13}H_{12}Cl_2O_4$.

Ethacrynic Acid Tablets

Etacrynic Acid Tablets

Ethacrynic Acid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethacrynic acid, $C_{13}H_{12}Cl_2O_4$.

Usual strength. 50 mg.

Identification

Mix a quantity of the powdered tablets containing 50 mg of Ethacrynic Acid with *0.1 M hydrochloric acid* and extract with two quantities, each of 40 ml, of *dichloromethane*. Dry the combined extracts with *anhydrous sodium sulphate*, filter and evaporate to dryness with the aid of gentle heat. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethacrynic acid IPRS* or with the reference spectrum of ethacrynic acid.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in a mixture of 99 volumes of *methanol* and 1 volume of *1 M hydrochloric acid* shows a well defined absorption maximum at 270 nm and a shoulder at 285 nm; absorbance at 270 nm, 0.55 to 0.60.

C. To 25 mg add 2 ml of *1 M sodium hydroxide* and heat in a water-bath for 5 minutes, cool, add 0.25 ml of *sulphuric acid (50 per cent v/v)* and 0.5 ml of a 10 per cent w/v solution of chromotropic acid sodium salt and add cautiously 2 ml of *sulphuric acid*; a deep violet colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml 0.1M phosphate buffer prepared by dissolving 13.6 g of *monobasic potassium phosphate* and 92.2 ml of *1M sodium hydroxide* with *water* to 1000 ml,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with medium, if necessary, at the maximum at about 277 nm (2.4.7). Calculate the content of $C_{13}H_{12}Cl_2O_4$ in the medium from the absorbance obtained from a solution of known concentration of *ethacrynic acid IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{13}H_{12}Cl_2O_4$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *chloroform*, 50 volumes of *ethyl acetate* and 20 volumes of *glacial acetic acid*.

Test solution. Shake a quantity of the powdered tablets containing 0.2 g of Ethacrynic Acid with 10 ml of *ethanol (95 per cent)* and filter.

Reference solution (a). Dilute 3 volumes of the test solution to 200 volumes with *ethanol (95 per cent)*.

Reference solution (b). Dilute 1 volume of the test solution to 200 volumes with *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Weigh and powder 20 tablets. Disperse a quantity of the powdered tablets containing about 50 mg of Ethacrynic Acid with 0.5 ml of *glacial acetic acid* and 50.0 ml of *acetonitrile* for 15 minutes and filter.

Test solution (b). Prepare in the same manner as test solution (a) but using 0.5 ml of *glacial acetic acid*, 45 ml of *acetonitrile* and 5.0 ml of a 0.15 per cent w/v solution of *propyl hydroxybenzoate* (internal standard) in *acetonitrile*.

Reference solution. Dissolve 50 mg of *ethacrynic acid IPRS* in 5.0 ml of the internal standard solution and dilute to 50.0 ml with a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*.

Chromatographic system

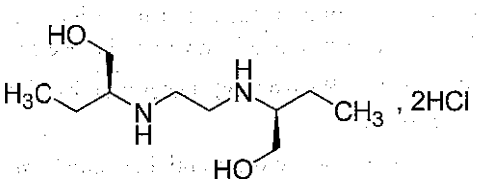
- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *acetonitrile* and 1 volume of *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{12}Cl_2O_4$ in the tablets.

Ethambutol Hydrochloride

Ethambutol Dihydrochloride



$C_{10}H_{24}N_2O_2,2HCl$ Mol. Wt. 277.2

Ethambutol Hydrochloride is (*S*,*S*)-*N*,*N*′-ethylenebis(2-aminobutan-1-ol) dihydrochloride.

Ethambutol Hydrochloride contains not less than 97.0 per cent and not more than 101.0 per cent of $C_{10}H_{24}N_2O_2,2HCl$, calculated on the dried basis.

Category. Antituberculosis.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethambutol hydrochloride IPRS* or with the reference spectrum of ethambutol hydrochloride.

B. In the test for 2-Aminobutanol, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. A 5.0 per cent w/v solution gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 3.4 to 4.0, determined in a 2.0 per cent w/v solution.

Specific optical rotation (2.4.22). +6.0° to +6.6°, determined in a 10.0 per cent w/v solution.

2-Aminobutanol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 75 volumes of *methanol*, 15 volumes of *water* and 10 volumes of *strong ammonia solution*.

Test solution (a). Dissolve 0.5 g of the substance under examination in 10.0 ml of *methanol*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *2-aminobutanol IPRS* in *methanol*.

Reference solution (b). A 0.5 per cent w/v solution of *ethambutol hydrochloride IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat at 110° for 10 minutes, cool, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Meso ethambutol (RS isomer). Determine by either of the following methods.

Method A. Determine by differential scanning calorimetry (DSC) (2.4.31).

Test preparation: Weigh between 4 and 6 mg of the sample in the 40 µl aluminium DSC crucible. Carry out the test by heating at a rate of 10° per minute from 25° to 250°, under nitrogen purging (20 ml/min) and record the thermogram. Observe the endotherms at 42° ± 2° and 77° ± 2° corresponding to the transitions of the RS isomer and SS isomer, respectively. There should not be any endothermic peak at 42° ± 2° in the thermogram.

Method B. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solution.

Test solution. Suspend 4.0 mg of the substance under examination in 4.0 ml of *acetonitrile* and 100 µl of *triethylamine*. Stir the mixture with the aid of ultrasound for 5 minutes. Add 15 µl of *R-(+)-phenyl isocyanate* and heat the mixture for 20 minutes at 70° in a water-bath.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

Reference solution (b). Suspend 4.0 mg of *ethambutol for system suitability IPRS* (containing RS isomer) 4.0 ml of *acetonitrile* and 100 µl of *triethylamine*. Mix the mixture with the aid of ultrasound for 5 minutes. Add 15 µl of *R-(+)-phenyl isocyanate* and heat the mixture for 20 minutes at 70° in a water-bath.

Chromatographic system

- a column 10 cm x 4.6 mm, packed with octadecylsilane bonded to silica (3 µm),
- column temperature. 40°,
- mobile phase: A. a mixture of equal volumes of *methanol* and *water*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	71	29
30	71	29
35	0	100
37	0	100
38	71	29

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to ethambutol and the RS isomer is not less than 4.0 and the relative retention of RS isomer with reference to ethambutol (retention time about 14 min) is about 1.3.

Inject reference solution (a) and the test solution.

In the chromatogram obtained with the test solution, the area of the peak due to the RS isomer is not more than the area of the peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Diluent. Dissolve 1.4 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml of *water* and adjusted to pH 6.8 ± 0.05 with *orthophosphoric acid*.

Test solution. Dissolve 30.0 mg of the substance under examination in 100.0 ml of the diluent.

Reference solution. A 0.03 per cent w/v solution of *ethambutol hydrochloride IPRS* in the diluent.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm) (Such as Zorbax SB-CN),
- mobile phase: a mixture of equal volumes of a buffer consisting of 1 ml of *triethylamine* in sufficient *water* to produce 1000 ml, adjusted to pH 7.0 with *orthophosphoric acid* and *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{24}N_2O_2,2HCl$.

Storage. Store protected from moisture.

Ethambutol Injection

Ethambutol Hydrochloride Injection

Ethambutol Injection is a sterile solution of Ethambutol hydrochloride in water for injection.

Ethambutol Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethambutol hydrochloride, $C_{10}H_{24}N_2O_2,2HCl$.

Usual strength. 100 mg per ml.

Description. A clear, colourless solution.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

B. When examined in the range 400 nm to 750 nm (2.4.7), a 0.04 per cent w/v solution exhibits absorption maxima at 620 nm.

Tests

pH (2.4.24). 3.5 to 4.5.

Appearance of solution. A 10.0 per cent w/v solution of Ethambutol in *water* is clear (2.4.1) and colourless (2.4.1).

2-Aminobutanol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 75 volumes of *methanol*, 15 volumes of *water* and 10 volumes of *ammonia*.

Test solution. Dilute a volume of injection containing 0.5 g of Ethambutol Hydrochloride to 10.0 ml with *methanol*.

Reference solution. A 0.05 per cent w/v solution of *2- aminobutanol IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat at 110° for 5 minutes, cool, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

Pyrogens (2.2.8). Complies with the test for pyrogens.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

NOTE— Use freshly prepared solutions.

Test solution. Dilute a volume of injection with *water* to obtain 0.04 per cent w/v solution of Ethambutol Hydrochloride.

Reference solution. A 0.04 per cent w/v solution of *ethambutol hydrochloride IPRS* in *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of a buffer solution prepared by diluting 2 ml of *triethylamine* to 1000 ml with *water*, adjusted to pH 7.0 with *orthophosphoric acid* and *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{24}N_2O_2,2HCl$ in the injection.

Storage. Store protected from light and moisture.

Ethambutol Tablets

Ethambutol Hydrochloride Tablets

Ethambutol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ethambutol hydrochloride, $C_{10}H_{24}N_2O_2,2HCl$.

Usual strengths. 200 mg; 400 mg.

Identification

A. Extract a quantity of the powdered tablets containing 50 mg of Ethambutol Hydrochloride with 5 ml of *methanol*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethambutol hydrochloride IPRS* or with the reference spectrum of ethambutol hydrochloride.

B. Shake a quantity of the powdered tablets containing 0.1 g of Ethambutol Hydrochloride with 10 ml of *water*, filter, and to the filtrate add 2 ml of a 1 per cent w/v solution of *copper sulphate* and 1 ml of *1 M sodium hydroxide*; a distinct blue colour is produced.

Tests

2-Aminobutanol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 75 volumes of *methanol*, 15 volumes of *water* and 10 volumes of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 0.5 g of Ethambutol Hydrochloride for 5 minutes with sufficient *methanol* to produce 10 ml and filter.

Reference solution. A 0.05 per cent w/v solution of *2-aminobutanol IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat at 110° for 5 minutes, cool, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium. 900 ml of freshly distilled *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute suitably with *water* to produce a solution containing about 0.030 per cent w/v of ethambutol hydrochloride. Using the resulting solution as the test solution carry out the procedure described under Assay.

Calculate the content of $C_{10}H_{24}N_2O_2,2HCl$.

Q. Not less than 75 per cent of the stated amount of $C_{10}H_{24}N_2O_2,2HCl$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE —Prepare the following solutions freshly.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 30 mg of Ethambutol Hydrochloride, add 50 ml of *water* and shake for about 15 minutes and add sufficient *water* to produce 100.0 ml. Filter and discard the first 10 ml of the filtrate. Use the clear filtrate.

Reference solution solution. A 0.03 per cent w/v solution of *ethambutol hydrochloride IPRS* in *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (5 µm) (Such as Zorbax SB-CN),
- column temperature: 30°,
- mobile phase: a mixture of equal volumes of a buffer consisting of 1 ml of *triethylamine* in sufficient *water* to produce 1000 ml adjusted to pH 7.0 with *orthophosphoric acid*, and *acetonitrile*,
- flow rate: 1 ml per minute
- spectrophotometer set at 200 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{24}N_2O_2,2HCl$ in the tablets.

Ethambutol and Isoniazid Tablets

Ethambutol Hydrochloride and Isoniazid Tablets

Ethambutol and Isoniazid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of ethambutol hydrochloride, $C_{10}H_{24}N_2O_2.2HCl$ and isoniazid, $C_6H_7N_3O$.

Usual strength. Ethambutol 400 mg and isoniazid 150 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due

to *ethambutol hydrochloride IPRS* in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to *isoniazid IPRS* in the chromatogram obtained with the reference solution.

Tests

2-Aminobutanol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel* G.

Mobile phase. A mixture of 75 volumes of *methanol*, 15 volumes of *water* and 10 volumes of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 0.5 g of ethambutol hydrochloride for 5 minutes with sufficient *methanol* to produce 10 ml and filter.

Reference solution. A 0.05 per cent w/v solution of *2-aminobutanol IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat at 110° for 5 minutes, cool, spray with *ninhydrin solution* and heat at 110° for 5 minutes. Any spot corresponding to 2-aminobutanol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 10 ml of the filtrate.

On the filtrate determine by liquid chromatography (2.4.14).

For Ethambutol Hydrochloride —

Test solution. Dilute the filtrate to obtain 0.044 per cent w/v solution in the dissolution medium.

Reference solution. A 0.044 per cent w/v solution of *ethambutol hydrochloride IPRS* in the dissolution medium.

Determine the content of $C_{10}H_{24}N_2O_2.2HCl$ by the procedure given under Assay of Ethambutol hydrochloride.

Calculate the content of $C_{10}H_{24}N_2O_2.2HCl$ in the medium.

For Isoniazid — Determine the amount of $C_6H_7N_3O$ dissolved by measuring the absorbance of the filtrate, suitably diluted with the dissolution medium to obtain a solution containing about 0.015 mg of isoniazid per ml, at the maximum at about 263 nm (2.4.7). Calculate the content of $C_6H_7N_3O$ in the medium from the absorbance obtained by repeating the determination using a 0.0015 per cent w/v solution of *isoniazid IPRS* in place of the filtrate.

Q. Not less than 75 per cent of the stated amount of $C_{10}H_{24}N_2O_2.2HCl$ and $C_6H_7N_3O$.

Other tests. Comply with the tests stated under Tablets.

Assay. *For isoniazid* — Determine by liquid chromatography (2.4.14).

Diluent. Dissolve 1.4 g of *disodium hydrogen orthophosphate anhydrous* in *water*, adjusted to pH 6.8 ± 0.05 with *dilute phosphoric acid* and add sufficient *water* to produce 1000 ml.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 40 mg of Isoniazid, dissolve in 50.0 ml of *methanol* and dilute to 500.0 ml with the diluent.

Reference solution. Weigh 40 mg of *isoniazid IPRS*, dissolve in 50.0 ml of *methanol* and dilute to 500.0 ml with the diluent.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Intersil ODS-3),
- column temperature: 30°,
- mobile phase: 96 volumes of buffer solution pH 6.8 prepared by dissolving 1.4 g *disodium hydrogen orthophsophate anhydrous* in 1000 ml of *water*, adjusted to pH 6.8 ± 0.05 with dilute *orthophosphoric acid* and 4 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the isoniazid peak is not more than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_6H_7N_3O$ in the tablets.

For ethambutol hydrochloride — Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 60 mg of Ethambutol Hydrochloride and dissolve in 100.0 ml of the diluent.

Reference solution. A 0.06 per cent w/v solution of *ethambutol hydrochloride IPRS* in the diluent.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (5 µm) (Such as Zorbax SB CN),
- mobile phase: a mixture of 50 volumes of buffer pH 7.0 prepared by mixing 1 ml of *triethylamine* in 1000 ml of *water* the pH of which is adjusted to 7.0 ± 0.05 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,

- flow rate: 1 ml per minute,
- spectrophotometer set at 200 nm;
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from ethambutol peak is not more than 1500 theoretical plates, the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{24}N_2O_2.2HCl$ in the tablets.

Storage. Store protected from moisture.

Ethanol

Absolute Alcohol; Absolute Ethanol; Dehydrated Alcohol

CH_3CH_2OH Mol. Wt. 46.1

Ethanol contains not less than 99.0 per cent v/v of C_2H_6O.

Category. Pharmaceutical aid (solvent).

Description. A clear, colourless, mobile and volatile liquid; hygroscopic. Readily volatilises even at low temperature; boils at 78°; flammable, burning with a blue, smokeless flame.

Identification

A. Mix 0.25 ml in a small beaker with 1 ml of *potassium permanganate solution* and 0.25 ml of *dilute sulphuric acid* and cover the beaker immediately with a filter paper moistened with a solution freshly prepared by dissolving 0.1 g of *sodium nitroprusside* and 0.5 g of *piperazine hydrate* in 5 ml of *water*; an intense blue colour is produced on the filter paper, the colour becoming lighter after a few minutes.

B. To 5 ml of a 0.5 per cent v/v solution add 1 ml of *1 M sodium hydroxide* followed by slow addition of 2 ml of *iodine solution*; the odour of iodoform develops and a yellow precipitate is produced.

Tests

Relative density (2.4.29). 0.7871 to 0.7902, determined at 25°.

Appearance of solution. Dilute 5.0 ml to 100.0 ml with *water*. The solution is clear (2.4.1). Cool to 10° for 30 minutes; the solution remains clear.

Acidity or alkalinity. To 20 ml add 0.25 ml of *phenolphthalein solution*; the solution remains colourless and requires not more than 0.2 ml of *0.1 M sodium hydroxide* to produce a pink colour.

Methanol. To 1 drop, add 1 drop of water, 1 drop of *dilute phosphoric acid* and 1 drop of *potassium permanganate solution*. Mix, allow to stand for 1 minute and add *sodium bisulphite solution* dropwise until the permanganate colour is discharged. If a brown colour remains, add 1 drop of *dilute phosphoric acid*. To the colourless solution add 5 ml of freshly prepared *chromotropic acid solution* and heat on a water-bath at 60° for 10 minutes; no violet colour is produced.

Foreign organic substances. Clean a glass-stoppered cylinder thoroughly with *hydrochloric acid*, rinse with *water* and finally rinse with the substance under examination. Put 20 ml in the cylinder, cool to about 15° and then add from a carefully cleaned pipette 0.1 ml of *0.1 M potassium permanganate*. Mix at once by inverting the stoppered cylinder and allow to stand at 15° for 5 minutes; the pink colour does not entirely disappear.

2-Propanol and 2-methyl-2-propanol. To 1 ml add 3 ml of *water* and 10 ml of *mercuric sulphate solution* and heat in a boiling water-bath; no precipitate is formed within 3 minutes.

Aldehydes. Not more than 10 ppm, determined by the following method. To 5.0 ml add 5 ml of *water* and 1 ml of *decolorised magenta solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that produced by treating in the same manner 5.0 ml of a 0.001 per cent w/v solution of redistilled *acetaldehyde* in *aldehyde-free ethanol (95 per cent)*.

Benzene and related substances. Determine by gas chromatography (2.4.13).

Test solution (a). The substance under examination.

Test solution (b). A 0.03 per cent v/v solution of *4-methylpentan-2-ol* in test solution (a).

Reference solution (a). A 0.02 per cent v/v solution of *anhydrous methanol* in test solution (a).

Reference solution (b). A 0.1 per cent v/v solution, each of, *anhydrous methanol* and *acetaldehyde* in test solution (a). Dilute 0.1 ml of the solution to 10.0 ml with test solution (a).

Reference solution (c). A 0.3 per cent v/v solution of *acetal* in test solution (a). Dilute 0.1 ml of the solution to 10.0 ml with test solution (a).

Reference solution (d). A 0.1 per cent v/v solution of *benzene* in test solution (a). Dilute 0.1 ml of the solution to 50.0 ml with test solution (a).

Chromatographic system

- a capillary column 30 m x 0.32 mm, packed with 6 per cent cyanopropylphenyl and 94 per cent of dimethyl polysiloxane (film thickness 1.8 µm),
- temperature
  column 40° for 0-12 minutes, 40° to 240° for 12-32 minutes and 240° for 32-42 minutes.

- inlet port at 200° and detector at 280°,
- flame ionization detector,
- split ratio: 1:20,
- flow 35 cm per second, using nitrogen as carrier gas,
- injection volume: 1 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to acetaldehyde (first peak) and methanol (second peak) is not less than 1.5.

Inject reference solution (a), (b), (c) and test solution (a). In the chromatogram obtained with the test solution (a), the area of any peak corresponding to methanol is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (200 ppm) and the sum of the contents of acetaldehyde and acetal, is not more than 10 ppm v/v, expressed as acetaldehyde.

Calculate the sum of the contents of acetaldehyde and acetal in parts per million v/v using the following expression:

$$\frac{10 \times A_E}{A_T - A_E} + \frac{30 \times C_E}{C_T - C_E} \times \frac{44.05}{118.2}$$

where, A_E = area of the peak due to acetaldehyde in the chromatogram obtained with test solution (a),

A_T = area of the peak due to acetaldehyde in the chromatogram obtained with reference solution (b),

C_E = area of the peak due to acetal in the chromatogram obtained with test solution (a),

C_T = area of the peak due to acetal in the chromatogramobtained with reference solution (c),

44.05 = molecular weight of acetaldehyde,

118.2 = molecular weight of acetal.

Content of benzene is not more than 2 ppm v/v, calculated in parts per million v/v using the following expression

$$\frac{2B_E}{B_T - B_E}$$

where, B_E = area of the peak due to benzene in the chromatogram obtained with test solution (a),

B_T = area of the peak due to benzene in the chromatogram obtained with reference solution (d).

The sum of areas of all other secondary peaks obtained with test solution (b) is not more than the area of 4-methylpentan-2-ol peak in the chromatogram obtained with test solution (b) (300 ppm). Ignore any peak with an area less than 0.03 times the area of 4-methylpentan-2-ol peak in the chromatogram obtained with test solution (b) (9 ppm).

Non-volatile matter. Evaporate 100.0 ml in a tared dish on a water-bath and dry the residue at 105°; the residue weighs not more than 5 mg.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution . A 1.0 per cent v/v solution of *1-propanol* in *water.*

Test solution. Dilute 1.0 ml of ethanol to 100.0 ml with internal standard solution.

Reference solution. A 1.0 per cent v/v solution of *ethanol* in internal standard solution.

Chromatographic system

- a capillary column 30 m × 0.25 mm, packed with 6.0 per cent cyanopropylphenyl and 94 per cent dimethylpolysiloxane (film thickness 1.4 µm) (Such as DB-624),
- temperature:
  column 50° for 2 minutes, 50° to 240° @ 20° per minute and hold at 240° for 2 minutes,
  inlet port 180° and detector at 260°,
- flame ionization detector,
- split ratio: 25:1,
- flow rate 0.5 ml per minutes, using nitrogen as carrier gas,
- injection volume: 1 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to ethanol and internal standard is not less than 2.0 and the relative standard deviation of peak area ratio due to ethanol and internal standard for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of C_2H_6O, using ratio of the peak area of ethanol to that of peak area of the internal standard.

Storage. Store in tightly-closed containers at a temperature not exceeding 30°, away from fire and protected from moisture.

Labelling. The label states that it is flammable.

Ethanol (95 per cent)

Alcohol (95 per cent)

Ethanol (95 per cent) is a mixture of Ethanol and Water.

Ethanol (95 per cent) contains not less than 94.0 per cent v/v and not more than 96.0 per cent v/v of C_2H_6O.

Category. Pharmaceutical aid (solvent); topical anti-infective.

Description. A clear, colourless, mobile and volatile liquid. It is readily volatilised even at low temperatures; boils at about 78°; flammable, burning with a blue, smokeless flame.

Identification

A. Mix 0.25 ml in a small beaker with 1 ml of *potassium permanganate solution* and 0.25 ml of *dilute sulphuric acid* and cover the beaker immediately with a filter paper moistened with a solution freshly prepared by dissolving 0.1 g of *sodium nitroprusside* and 0.5 g of *piperazine hydrate* in 5 ml of *water*; an intense blue colour is produced on the filter paper, the colour becoming lighter after a few minutes.

B. To 5 ml of a 0.5 per cent v/v solution add 1 ml of *1 M sodium hydroxide* followed by slow addition of 2 ml of *iodine solution*; the odour of iodoform develops and a yellow precipitate is produced.

Tests

Relative density (2.4.29). 0.8084 to 0.8104, determined at 25°.

Appearance of solution. Dilute 5.0 ml to 100.0 ml with *water*. The solution is clear (2.4.1). Cool to 10° for 30 minutes; the solution remains clear.

Acidity or alkalinity. To 20 ml add 0.25 ml of *phenolphthalein solution*; the solution remains colourless and requires not more than 0.2 ml of *0.1 M sodium hydroxide* to produce a pink colour.

Methanol. To 1 drop add 1 drop of *water*, 1 drop of *dilute phosphoric acid* and 1 drop of *potassium permanganate solution*. Mix, allow to stand for 1 minute and add *sodium bisulphite solution* dropwise until the permanganate colour is discharged. If a brown colour remains, add 1 drop of *dilute phosphoric acid*. To the colourless solution add 5 ml of freshly prepared *chromotropic acid solution* and heat on a water-bath at 60° for 10 minutes; no violet colour is produced.

Foreign organic substances. Clean a glass-stoppered cylinder thoroughly with *hydrochloric acid*, rinse with *water* and finally rinse with the substance under examination. Put 20 ml in the cylinder, cool to about 15° and then add from a carefully cleaned pipette 0.1 ml of *0.1 M potassium permanganate*. Mix at once by inverting the stoppered cylinder and allow to stand at 15° for 5 minutes; the pink colour does not entirely disappear.

2-Propanol and 2-methyl-2-propanol. To 1 ml add 3 ml of *water* and 10 ml of *mercuric sulphate solution* and heat in a boiling water-bath; no precipitate is formed within 3 minutes.

Aldehydes. Not more than 10 ppm, determined by the following method. To 5.0 ml add 5 ml of *water* and 1 ml of *decolorised magenta solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that produced by treating in the same manner 5 ml of a 0.001 per cent w/v solution of redistilled *acetaldehyde* in *aldehyde-free ethanol (95 per cent)*.

Benzene and related substances. Determine by gas chromatography (2.4.13).

Test solution (a). The substance under examination.

Test solution (b). A 0.03 per cent v/v solution of *4-methylpentan-2-ol* in test solution (a).

Reference solution (a). A 0.02 per cent v/v solution of *anhydrous methanol* in test solution (a).

Reference solution (b). A 0.1 per cent v/v solution, each of *anhydrous methanol* and *acetaldehyde* in test solution (a). Dilute 0.1 ml of the solution to 10.0 ml with test solution (a).

Reference solution (c). A 0.3 per cent v/v solution of *acetal* in test solution (a). Dilute 0.1 ml of the solution to 10.0 ml with test solution (a).

Reference solution (d). A 0.1 per cent v/v solution of *benzene* in test solution (a). Dilute 0.1 ml of the solution to 50.0 ml with test solution (a).

Chromatographic system

- a capillary column 30 m x 0.32 mm, packed with 6 per cent cyanopropylphenyl and 94 per cent of dimethyl polysiloxane (film thickness 1.8 µm),
- temperature
  column 40° for 0-12 minutes, 40° to 240° for 12-32 minutes and 240° for 32-42 minutes.
- inlet port at 200° and detector at 280°,
- flame ionization detector,
- split ratio: 1:20,
- flow 35 cm per second, using nitrogen as carrier gas,
- injection volume: 1 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to acetaldehyde (first peak) and methanol (second peak) is not less than 1.5.

Inject reference solution (a), (b), (c) and test solution (a). In the chromatogram obtained with the test solution (a), the area of any peak corresponding to methanol is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (200 ppm) and the sum of the contents of acetaldehyde and acetal, is not more than 10 ppm v/v, expressed as acetaldehyde.

Calculate the sum of the contents of acetaldehyde and acetal in parts per million v/v using the following expression:

$$\frac{10 \times A_E}{A_T - A_E} + \frac{30 \times C_E}{C_T - C_E} \times \frac{44.05}{118.2}$$

Where, A_E = area of the peak due to acetaldehyde in the chromatogram obtained with test solution (a),

A_T = area of the peak due to acetaldehyde in the chromatogram obtained with reference solution (b),

C_E = area of the peak due to acetal in the chromatogram obtained with test solution (a).

C_T = area of the peak due to acetal in the chromatogramobtained with reference solution (c),
44.05 = molecular weight of acetaldehyde,
118.2 = molecular weight of acetal.

Content of benzene is not more than 2 ppm v/v, calculated in parts per million v/v using the following expression

$$\frac{2B_E}{B_T - B_E}$$

Where, B_E = area of the peak due to benzene in the chromatogram obtained with test solution (a),
B_T = area of the peak due to benzene in the chromatogram obtained with reference solution (d).

The sum of areas of all other secondary peaks obtained with test solution (b) is not more than the area of 4-methylpentan-2-ol peak in the chromatogram obtained with test solution (b) (300 ppm). Ignore any peak with an area less than 0.03 times the area of 4-methylpentan-2-ol peak in the chromatogram obtained with test solution (b) (9 ppm).

Fusel oil constituents. Place 25 ml in a porcelain dish protected from dust and allow the liquid to evaporate on a water-bath until a little of the liquid remains. Remove the dish from the water-bath and allow the liquid to evaporate at room temperature till the dish is almost dry. No foreign odour is perceptible. Add 1 ml of *sulphuric acid*; no red or brown colour is produced.

Non-volatile matter. Evaporate 100.0 ml in a tared dish on a water-bath and dry the residue at 105°; the residue weighs not more than 5 mg.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. A 1.0 per cent v/v solution of *1-propanol* in *water*.

Test solution. Dilute 1.0 ml of Ethanol (95 per cent) to 100.0 ml with internal standard solution.

Reference solution. A 1.0 per cent v/v solution of *ethanol* in internal standard solution.

Chromatographic system
- a capillary column 30 m × 0.25 mm, packed with 6.0 per cent cyanopropylphenyl and 94 per cent dimethylpolysiloxane (film thickness 1.4 µm) (Such as DB-624),
- temperature:
  column 50° for 2 minutes, 50° to 240° @ 20° per minute and hold at 240° for 2 minutes,
  inlet port 180° and detector at 260°,
- flame ionization detector,
- split ratio: 25:1,
- flow rate 0.5 ml per minutes, using nitrogen as carrier gas,
- injection volume: 1 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to ethanol and internal standard is not less than 2.0 and the relative standard deviation of peak area ratio due to ethanol and internal standard for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

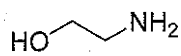
Calculate the content of C_2H_6O, using ratio of the peak area of ethanol to that of peak area of the internal standard.

Storage. Store in tightly-closed containers at a temperature not exceeding 30° and away from fire.

Labelling. The label states that it is flammable.

Ethanolamine

Monoethanolamine



C_2H_7NO Mol. Wt. 61.1

Ethanolamine is 2-aminoethanol.

Ethanolamine contains not less than 98.0 per cent and not more than 100.5 per cent of C_2H_7NO.

Category. Sclerosing agent.

Description. A clear, colourless or pale yellow liquid.

Identification

A. To 0.1 ml add 0.3 g of *picric acid* and 1 ml of *water* and evaporate to dryness on a water-bath. The melting point of the residue (2.4.24), after recrystallisation from *ethanol (95 per cent)* and drying at 105°, is about 160°.

B. When freshly distilled the second half of the distillate freezes at about 10°.

C. It is alkaline to litmus solution.

Tests

Refractive index (2.4.27). 1.453 to 1.459.

Weight per ml (2.4.29). 1.014 to 1.023 g.

Related substances. Determine by gas chromatography (2.4.13).

Solution A. A 0.1 per cent w/v solution of *3-aminopropan-1-ol* (internal standard) in *dichloromethane*.

Test solution. A 10.0 per cent w/v solution of the substance under examination in solution A. To 0.5 ml of the solution, add

0.5 ml of *trifluoroacetic anhydride*, mix and allow to stand for 10 minutes.

Reference solution. A solution containing 0.05 per cent w/v of *ethanolamine* and 0.1 per cent w/v each of *diethanolamine* and *triethanolamine* in solution A. To 0.5 ml of the solution, add 0.5 ml of *trifluoroacetic anhydride*, mix and allow to stand for 10 minutes.

Chromatographic system

- a capillary column 25 m x 0.22 mm packed with dimethylpolysiloxane (film thickness 0.25 µm),
- column temperature: 80° for 2 minutes, then increase to 200° at a rate of 8° per minute and maintain this temperature for 10 minutes,
- Inlet port at 240° and detector at 250°,
- flame ionization detector,
- split ratio: 1:40,
- flow rate: use helium or nitrogen as the carrier gas at 1.0 ml per minute with a flow rate of the make up gas of 20 ml per minute.

The peaks eluting after the solvent peak in order of emergence are due to (a) ethanolamine, (b) 3-aminopropan-1-ol, (c) diethanolamine and (d) triethanolamine.

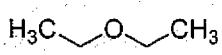
Inject 1 µl of the reference solution and the test solution.In the chromatogram obtained with the test solution calculate the content of diethanolamine and triethanolamine using the ratios of the peaks and by reference to the corresponding peaks in the chromatogram obtained with the reference solution. The content of diethanolamineand triethanolamine is not more than 1.0 per cent individually.

Calculate the content of other impurities using the ratios of the peaks and by reference to the peak due to ethanolamine. The content of any other impurity is not more than 0.5 per cent and the sum of the contents of all the impurities is not more than 2.0 per cent.

Assay. Dissolve 2.5 g in 50 ml of *1 M hydrochloric acid* and titrate the excess of acid with *1 M sodium hydroxide* using *methyl red solution* as indicator. Carry out a blank titration.

1 ml of *1 M hydrochloric acid* is equivalent to 0.06108 g of C_2H_7NO.

Anaesthetic Ether



$C_4H_{10}O$ Mol. Wt. 74.1

Anaesthetic Ether is diethyl ether to which a suitable non-volatile stabiliser in a proportion not greater than 0.002 per cent w/v may have been added.

Category. General anaesthetic.

Description. A clear, colourless, very mobile liquid; highly flammable.

NOTE — It is absolutely essential that a preservative of the type of sodium pyrogallate, hydroquinone or propyl gallate in suitable concentrations shall be added in Anaesthetic Ether intended for use in tropical climates unless the Anaesthetic Ether is stored in a copper container or in a container copper-plated internally. The preservative used and its concentration shall be declared on the label.

Tests

Relative density (2.4.29). 0.714 to 0.716, determined at 20°.

Boiling range (2.4.8). 34° to 35°.

CAUTION — It is dangerous to determine the boiling range if the sample does not comply with the test for peroxides.

Acidity. To 20 ml of *ethanol (95 per cent)* add 0.25 ml of *bromothymol blue solution* add dropwise *0.02 M sodium hydroxide* until the blue colour persists for 30 seconds. Add 25 ml of the substance under examination, shake and again add dropwise *0.2 M sodium hydroxide* until the blue colour reappears and persists for 30 seconds. Not more than 0.4 ml of *0.02 M sodium hydroxide* is required.

Peroxides. Place 8 ml of *potassium iodide and starch solution* in a 12-ml glass-stoppered cylinder of about 1.5 cm diameter. Fill completely with the substance under examination, insert the stopper, shake vigorously and allow to stand in the dark for 30 minutes; no colouration is produced.

Acetone and aldehydes. Place 2 ml of *alkaline potassium mercuri-iodide solution* in a 12-ml glass-stoppered cylinder of about 1.5 cm diameter and fill completely with the substance under examination, insert the stopper and shake vigorously for 15 seconds and set aside for 5 minutes, protected from light; no colour or turbidity, except for slight opalescence, is produced.

If the ether does not comply with the test, distil 40 ml *(after ensuring that it complies with the test for peroxides)* until only 5 ml remains and repeat the test using 10 ml of the distillate.

Foreign odour. Pour 10 ml in successive portions on to a clean filter paper and allow to evaporate spontaneously; no foreign odour is detectable at any stage of evaporation.

Non-volatile matter. Evaporate 50 ml in a tared dish on a water-bath and dry at 105° *(after ensuring that the sample complies with the test for peroxides)*; the residue weighs not more than 1.5 mg.

Methanol. To 10 ml, add 5 ml of *ethanol (20 per cent)* and 5 ml of *water*, in a separator, shake vigorously, set aside and allow the mixture to separate and draw off the lower layer. To 5 ml of

the lower layer add 2.0 ml of *potassium permanganate and phosphoric acid solution*, set aside for 10 minutes and add 2.0 ml of *oxalic acid* and *sulphuric acid solution* and 5 ml of *decolorised magenta solution*. Set aside for 30 minutes; no colour is produced.

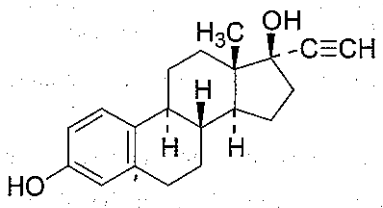
Water (2.3.43). Not more than 0.2 per cent, determined on 20.0 ml.

Storage. Store protected from light at a temperature not exceeding 30°. Ether remaining in a partly used container may deteriorate rapidly.

Labelling. The label states that (1) it is very flammable and should not be used near a naked flame; (2) the name and proportion of any stabiliser added.

Ethinyloestradiol

Ethinylestradiol



$C_{20}H_{24}O_2$ Mol. Wt. 296.4

Ethinylestradiol is 19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17β-diol.

Ethinyloestradiol contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{20}H_{24}O_2$, calculated on the dried basis.

Category. Oestrogen.

Description. A white or slightly yellowish-white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethinyloestradiol IPRS* or with the reference spectrum of ethinyloestradiol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to in the in the chromatogram obtained with reference solution (b).

C. Dissolve about 1 mg in 1 ml of *sulphuric acid*; an orange-red colour develops which exhibits a greenish fluorescence when examined under ultraviolet light at 365 nm. Add the solution to 10 ml of *water*; the colour changes to violet and a violet precipitate is produced.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *ethanol* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Specific optical rotation (2.4.22). −30.0° to −27.0°, determined at 20° in a 5.0 per cent w/v solution in *pyridine*.

Light absorption (2.4.7). Absorbance of a 0.01 per cent w/v solution in *ethanol (95 per cent)* at about 281 nm, 0.69 to 0.73.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (95 per cent)*.

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*.

Test solution (b). Dilute 5.0 ml of test solution (a) to 100.0 ml with the same solvent mixture.

Reference solution (a). Dilute 5.0 ml of test solution (b) to 25.0 ml with the same solvent mixture.

Reference solution (b). A 0.1 per cent w/v solution of *ethinyloestradiol IPRS* in the same solvent mixture.

Reference solution (c). A 0.02 per cent w/v solution of *estrone IPRS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, heat at 110° for 10 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat again at 110° for 10 minutes and examine under ultraviolet light at 365 nm. In the chromatogram obtained with test solution (a) any spot corresponding to estrone is not more intense than the spot in the chromatogram obtained with reference solution (c) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.2 g in 40 ml of *tetrahydrofuran*, add 5 ml of a 10 per cent w/v solution of *silver nitrate* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02964 g of $C_{20}H_{24}O_2$.

Storage. Store protected from light.

Ethinyloestradiol Tablets

Ethinylestradiol Tablets

Ethinyloestradiol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethinyloestradiol, $C_{20}H_{24}O_2$.

Usual strengths. 10 µg; 20 µg; 50 µg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (95 per cent)*.

Test solution. Shake a quantity of the powdered tablets containing 0.25 mg of Ethinyloestradiol with four quantities, each of 20 ml of *chloroform*, filter each extract in turn, evaporate the combined filtrates to dryness on a water-bath in a current of nitrogen and dissolve the residue in 0.25 ml of *chloroform*.

Reference solution. A 0.1 per cent w/v solution of *ethinyloestradiol IPRS* in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent v/v)*, heat at 110° for 10 minutes and examine under ultraviolet light at 365 nm and in daylight. By both methods of visualisation, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Triturate a quantity of the powdered tablets containing 0.1 mg of Ethinyloestradiol with 0.5 ml of *0.1 M sodium hydroxide* and 5 ml of *water*, allow to stand for 5 minutes, filter, acidify the filtrate with 0.15 ml of *sulphuric acid*, add 3 ml of *ether*, shake and allow to separate. Evaporate the ether layer to dryness and heat the residue on a water-bath for 5 minutes with 0.2 ml of *glacial acetic acid* and 2 ml of *phosphoric acid*; a pink colour with an intense orange fluorescence is produced.

Tests

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Finely crush one tablet, disperse in the mobile phase with the aid of ultrasound for 10 minutes and centrifuge. Dilute the supernatant liquid with the mobile phase to obtain a solution containing 0.0005 per cent w/v of Ethinyloestradiol.

Reference solution. A 0.0005 per cent w/v solution of *ethinylestradiol IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volume of *acetonitrile* and 40 volumes of *water*.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{24}O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 100 µg of Ethinylestradiol in the mobile phase with the aid of ultrasound for 10 minutes and centrifuge. Dilute the supernatant liquid with the mobile phase to obtain a solution containing 0.0005 per cent w/v of Ethinyloestradiol.

Reference solution. A 0.0005 per cent w/v solution of *ethinylestradiol IPRS* in the mobile phase.

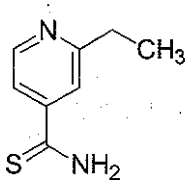
Use chromatographic system as described under Uniformity of content.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{24}O_2$ in the tablets.

Storage. Store protected from light.

Ethionamide



$C_8H_{10}N_2S$ Mol. Wt. 166.2

Ethionamide is 2-ethylpyridine-4-carbothioamide.

Ethionamide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_8H_{10}N_2S$, calculated on the dried basis.

Category. Antituberculosis.

Description. A yellow crystalline powder or small yellow crystals.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethionamide IPRS* or with the reference spectrum of ethionamide.

B. Dissolve about 10 mg in 5 ml of *methanol* and add 5 ml of *0.1 M silver nitrate*; a dark brown precipitate is produced.

C. Melting point (2.4.21). 158° to 164°.

Tests

Appearance of solution. Dissolve 0.5 g in 10 ml of *methanol*, heating to about 50° and allow to cool to room temperature. The solution is not more opalescent than opalescence standard OS2 (2.4.1).

Acidity. Dissolve 2.0 g in 20 ml of *methanol*, heating to about 50°, and add 20 ml of *water*. Cool slightly, shake until crystallisation occurs and allow to cool to room temperature. Add 60 ml of *water* and titrate with *0.1 M sodium hydroxide* using 0.2 ml of *cresol red solution* as indicator. Not more than 0.2 ml is required to change the colour of the solution to red.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution. Dissolve 25 mg of the *ethionamide IPRS* in 100 ml of the mobile phase. Dilute 1.0 ml of the solution to100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Intersil ODS-3),
- mobile phase: a mixture of 60 volumes of a buffer prepared by dissolving 2 ml of *triethylamine* in *water*, adjusted to pH 6.0 with *orthophosphoric acid* and diluting to 1000 ml, and 40 volumes of *acetonitrile* and filtered,
- flow rate: 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following solutions.

Test solution. Dissolve about 50 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve 50 mg of the *ethionamide IPRS* in 100.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Inject the reference solution. The test is not valid unless he column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{10}N_2S$.

Storage. Store protected from light and moisture.

Ethionamide Tablets

Ethionamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of ethionamide, $C_8H_{10}N_2S$.

Usual strength. 125 mg.

Identification

A. Extract a quantity of the powdered tablets containing 25 mg of Ethionamide with 5 ml of *methanol*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethionamide IPRS* or with the reference spectrum of ethionamide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium, filter and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 274 nm (2.7.4). Calculate the content of $C_8H_{10}N_2S$ from

the absorbance of a solution of known concentration of *ethionamide IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_8H_{10}N_2S$.

Related substances. Determine by liquid chromatography (2.4.14) as given under Assay using the following solutions.

Test solution. Disperse a quantity of the powder containing 50 mg of Ethionamide and dissolve in 100 ml of the mobile phase.

Reference solution. Dissolve 25 mg of the *ethionamide IPRS* in 100 ml of the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Ethionamide in 100.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve 50 mg of the *ethionamide IPRS* in 100 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Intersil ODS-3),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of buffer pH 6.0 prepared by mixing 2 ml of *triethylamine* to 1000 ml with *water* and adjusted to pH 6.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume: 20 µl.

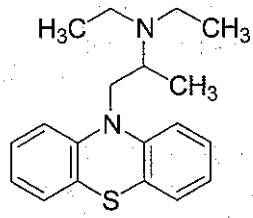
Inject the reference solution. The test is not valid unless the column efficiency in not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{10}N_2S$ in the tablets.

Storage. Store protected from light and moisture.

Ethopropazine Hydrochloride



$C_{19}H_{24}N_2S,HCl$ Mol. Wt. 348.9

Ethopropazine Hydrochloride is 10-[2-(diethylamino)-propyl]phenothiazine hydrochloride.

Ethopropazine Hydrochloride contains not less than 99.0 per cent and not more than 101.5 per cent of $C_{19}H_{24}N_2S,HCl$, calculated on the dried basis.

Category. Antiparkinsonian.

Description. A white or slightly creamy-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethopropazine hydrochloride IPRS* or with the reference spectrum of ethopropazine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at 252 nm and a less well-defined maximum at 303 nm; absorbance at 252 nm, about 0.42.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

D. It gives reaction (A) of chlorides (2.3.1).

Tests

Acidity or alkalinity. Dissolve 0.15 g in 50 ml of *carbon dioxide-free water* and add 0.15 ml of *methyl red solution*; the solution is yellow and not more than 0.2 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A freshly prepared mixture of equal volumes of *ether* and *ethyl acetate* saturated with *strong ammonia solution*.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Reference solution (b). A 0.5 per cent w/v solution of *ethopropazine hydrochloride IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.7 g, dissolve in 200 ml of *acetone*, add 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using 0.15 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03489 g of $C_{19}H_{24}N_2S,HCl$.

Storage. Store protected from light.

Ethopropazine Tablets

Ethopropazine Hydrochloride Tablets

Ethopropazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ethopropazine hydrochloride, $C_{19}H_{24}N_2S,HCl$.

Usual strength. 50 mg.

Identification

A. Extract a quantity of the powdered tablets containing 50 mg of Ethopropazine Hydrochloride with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethopropazine hydrochloride IPRS* or with the reference spectrum of ethopropazine hydrochloride.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. To a quantity of the powdered tablets containing 5 mg of Ethopropazine Hydrochloride add 5 ml of *sulphuric acid* and allow to stand for 5 minutes; a red colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A freshly prepared mixture of equal volumes of *ether* and *ethyl acetate* saturated with *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Ethopropazine Hydrochloride with 50 ml of *chloroform* for 15 minutes, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.2 per cent w/v solution of *ethopropazine hydrochloride IPRS* in *chloroform*.

Reference solution (b). A 0.002 per cent w/v solution of *ethopropazine hydrochloride IPRS* in *chloroform*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

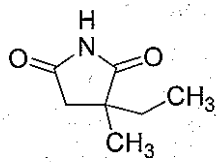
Other tests. Comply with the tests stated under Tablets.

Assay. *Protect the solution from light throughout the test.*

Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Ethopropazine Hydrochloride, extract with four quantities, each of 20 ml, of *ethanol (95 per cent)*. Filter and dilute the filtrate to 100.0 ml with *ethanol (95 per cent)*. Dilute 10.0 ml of the solution to 100.0 ml with *ethanol (95 per cent)*. Dilute 10.0 ml of the solution further to 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 252 nm (2.4.7). Calculate the content of $C_{19}H_{24}N_2S,HCl$, taking 845 as the specific absorbance at 252 nm.

Storage. Store protected from light.

Ethosuximide



$C_7H_{11}NO_2$ Mol. Wt. 141.2

Ethosuximide is (3*RS*)-3-ethyl-3-methypyrrolidine-2,5-dione.

Ethosuximide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_7H_{11}NO_2$, calculated on the anhydrous basis.

Category. Antiepileptic

Description. A white or almost white powder or waxy solid. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Melt a sufficient quantity at 50°, prepare a thin film between two previously warmed bromide plates and record the spectrum immediately. Compare the spectrum with that obtained with *ethosuximide IPRS* or with the reference spectrum of ethosuximide.

B. When examined in the range 230 nm to 360 nm (2.4.7), 0.1 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at 248 nm; about 0.85.

Tests

Appearance of solution. A 10 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

Cyanide. Dissolve 1.0 g in 10 ml of *ethanol (90 per cent)*, add 0.5 ml of *ferrous sulphate solution*, 1 ml of *2 M sodium hydroxide* and 0.1 ml of *ferric chloride solution*. Heat to boiling, cool and acidify using 3 ml of *1 M sulphuric acid*. After 15 minutes, there is no blue colour and no blue precipitate is produced.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.25 g of the substance under examination in mobile phase A and dilute to 10.0 ml with mobile phase A. store the solution at room temperature for at least 30 minutes before injection (*in situ* transformation of impurity B to impurity A).

Reference solution (a). A 0.05 per cent w/v solution of *ethosuximide impurity A ((2RS)-2-ethyl-2-methylbutanedionic acid) IPRS* in mobile phase A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A.

Reference solution (c). Mix 1.0 ml of reference solution (a) and 4.0 ml of the test solution.

Chromatographic system
- a stainless steel column 10 cm x 2.1 mm, packed with end-capped solid core octadecylsilane organosilica polymer compatible with 100 per cent aqueous mobile phase (2.6 µm),
- mobile phase: A. a buffer solution prepared by dissolving 15.6 g of *sodium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH 2.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*,

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 0.25 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 3 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
10	90	10
11	30	70
15	30	70
20	90	10

The relative retention time with reference to ethosuximide (retention time: about 4 minutes) for ethosuximide impurity A is about 1.7.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to ethosuximide and ethosuximide impurity A is not less than 3.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Dissolve 0.12 g in 20 ml of *dimethylformamide*. Titrate with *0.1M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01412 g of $C_7H_{11}NO_2$.

Storage. Store protected from light.

Ethosuximide Capsules

Ethosuximide Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ethosuximide, $C_7H_{11}NO_2$.

Usual strength. 250 mg.

Identification

A. Heat a quantity of the contents of the capsules containing 0.1 g of Ethosuximide with 0.2 g of *resorcinol* and 0.1 ml of *sulphuric acid* at 140° for 5 minutes, add 5 ml of *water*, make alkaline with *5 M sodium hydroxide* and add 0.2 ml to a large volume of *water;* a bright green fluorescence is produced.

B. Shake a quantity of the contents of the capsules containing 0.25 g of Ethosuximide with 80 ml of *ethanol (95 per cent)* for a few minutes, add sufficient *ethanol (95 per cent)* to produce 100 ml, mix and filter. Dilute 20 ml of the filtrate to 100 ml with *ethanol (95 per cent)*. Absorbance of the resulting solution at the maximum at about 248 nm, about 0.43 (2.4.7).

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the contents of the capsules containing 0.2 g of Ethosuximide and dissolve in 30 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, using a 0.1 per cent w/v solution of *azo violet* in *dimethylformamide* as indicator. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01412 g of $C_7H_{11}NO_2$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Ethosuximide Syrup

Ethosuximide Oral Solution

Ethosuximide Syrup is a solution of Ethosuximide in a suitable flavoured vehicle.

Ethosuximide Syrup contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ethosuximide, $C_7H_{11}NO_2$.

Usual strength. 250 mg in 5 ml.

Identification

A. Extract a quantity of the syrup containing 0.5 g of Ethosuximide with two quantities, each of 30 ml, of *chloroform*, filter the combined extracts through a plug of cotton and evaporate the filtrate to dryness. Heat 100 mg of the residue with 0.2 g of *resorcinol* and 0.1 ml of *sulphuric acid* at 140° for 5 minutes, cool, add 5 ml of *water*, make alkaline with *5 M sodium hydroxide* and add 0.2 ml to a large volume of *water*; a bright green fluorescence is produced.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Oral liquids.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). Add 10 ml of *water* and 2 g of *sodium bicarbonate* to a weighed quantity of the syrup containing about 0.25 g of Ethosuximide and extract with five quantities, each of 25 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. To the combined extracts add 10 ml of a 3.0 per cent w/v solution of *dimethyl phthalate* (internal standard) in *chloroform*, shake with 10 g of *anhydrous sodium sulphate* and filter.

Test solution (b). Prepare in the same manner as test solution (a) but omit the internal standard.

Reference solution. Add 2 ml of the internal standard solution to 25.0 ml of a 0.2 per cent w/v solution of *ethosuximide IPRS* in *chloroform*.

Chromatographic system

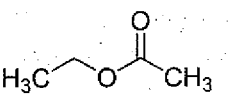
- a glass column 1.5 m x 4 mm, packed with acid-washed silanised diatomaceous support (80 to 100 mesh) impregnated with 3 per cent w/w of cyanopropylmethyl phenyl methyl silicone fluid (OV-225) of cyanopropylmethyl phenyl methyl silicone fluid (Such as OV-225),
- temperature:
  column.165°,
  inlet port and detector. 240°,
- flow rate: 30 ml per minute of the carrier gas.

Inject 2 µl of the reference solution, test solution (a) and (b).

Determine the weight per ml of the syrup (2.4.29), and calculate the content of $C_7H_{11}NO_2$, weight in volume.

Storage. Store at a temperature not exceeding 30°.

Ethyl Acetate



$C_4H_8O_2$ Mol. Wt. 88.1

Ethyl Acetate is Acetic acid, ethyl ester.

Ethyl Acetate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_4H_8O_2$.

Category. Excipient.

Description. A transparent, colourless liquid.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethyl acetate IPRS* or with the reference spectrum of ethyl acetate.

Tests

Specific gravity (2.4.29). 0.894 to 0.898.

Acidity. To 2.0 ml, add 10 ml of neutralized *ethanol*, add 2 drops of *phenolphthalein* solution. Neutralize with *0.1M sodium hydroxide*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Readily carbonizable substances. To 2.0 ml, add 10.0 ml of *sulphuric acid* to form separate layers. No dark zone is developed within 15 minutes.

Limit of nonvolatile residue. Not more than 0.02 per cent, determined by evaporating 100 g of Ethyl Acetate to dryness in a tared porcelain dish on a steam-bath and dry at 105° for 1 hour.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 1.6 g of Ethyl Acetate in 10.0 ml of *N,N-dimethylacetamide*.

Reference solution (a). A solution containing 0.016 per cent w/v, each of, *acetaldehyde* and *methanol*, 16.0 per cent w/v of *ethyl acetate IPRS* and 0.16 per cent w/v of *methyl ethyl ketone IPRS* in *N,N-dimethylacetamide*.

Reference solution (b). A solution containing 0.016 per cent w/v, each of, *acetaldehyde*, *ethyl acetate IPRS* and *1-ethoxy-2-methylpropane IPRS* in *N,N-dimethylacetamide*.

Reference solution (c). A solution containing 0.016 per cent w/v, each of, *methanol, methyl acetate*, and *methyl isobutyrate* in *N,N-dimethylacetamide* (For identification of Methyl compounds).

Reference solution (d). Dilute 5.0 ml of reference solution (b) to 10.0 ml with *N,N-dimethylacetamide*.

Chromatographic system

- a fused-silica capillary column 60 m x 0.32 mm coated with 6 per cent cyanopropylphenyl- 94 per cent dimethylpolysiloxane (film thickness 1.8 µm) (Such as DB 624),
- temperature:
  column. 40° for 15 minutes, 40° to 200° @ 12° per minute and hold at 200° for 2 minutes,
- inlet port at 210° and detector at 250°,
- flame ionisation detector,
- flow rate: 3 ml per minute using nitrogen as carrier gas,
- split ratio: 30:1,
- injection volume: 1 µl.

Name	Relative retention time
Acetaldehyde	0.29
Methanol	0.31
Methyl ethyl ketone	0.97
Ethyl acetate	1.0
1-ethoxy-2-methylpropane	1.1

Inject reference solution (a), (b) and (d). The test is not valid unless the resolution between the peaks due to acetaldehyde and methanol is not less than 2.0 and between the peaks due to methyl ethyl ketone and ethyl acetate is not less than 2.0 in the chromatogram obtained with reference solution (a), the relative standard deviation for replicate injections is not more than 5.0 per cent and the tailing factor is not more than 1.5 for acetaldehyde, ethyl acetate and 1-ethoxy-2-methylpropane peaks in the chromatogram obtained with reference solution (b) and signal-to-noise ratio for acetaldehyde, ethyl acetate, and 1-ethoxy-2-methylpropane peaks is not less than 20 in the chromatogram obtained with reference solution (d).

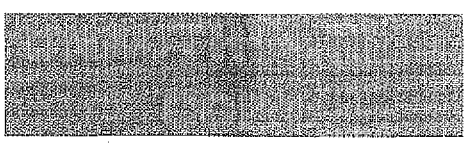
Inject reference solution (c) to identify the peaks due to methanol, methyl acetate and methyl isobutyrate.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to acetaldehyde and 1-ethoxy-2-methylpropane, each of, is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the sum of the areas of the peaks corresponding to methyl compound (methanol, methyl acetate and methyl isobutyrate) multiplied by correction factor 0.1 is not more than the area of the ethyl acetate peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks other than acetaldehyde, 1-ethoxy-2-methylpropane and methyl compounds is not more than 3 times the area of the ethyl acetate peak in the chromatogram obtained with reference solution (b) (0.3 per cent).

Bacterial endotoxins (2.2.3). If labeled for use in preparing parenteral dosage forms, it also meets the following requirements. The level of bacterial endotoxins is such that the requirement in the relevant dosage form monographs in which Ethyl Acetate is used can be met. Where the label states that Ethyl Acetate must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph in which Ethyl Acetate is used can be met.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 50 mg of Ethyl Acetate in 25.0 ml of *N,N-dimethylacetamide*.



Reference solution (a). A solution containing 0.2 per cent w/v of *ethyl acetate IPRS* and 0.002 per cent w/v of *methyl ethyl ketone IPRS* in *N,N-dimethylacetamide.*

Reference solution (b). A 0.2 per cent w/v solution of *ethyl acetate IPRS* in *N,N-dimethylacetamide.*

Use chromatographic system as described under Related substances.

The relative retention time with reference to ethyl acetate for methyl ethyl ketone is about 0.97.

Inject reference solution (a), (b). The test is not valid unless the resolution between the peaks due to methyl ethyl ketone and ethyl acetate is not less than 2.0 in the chromatogram obtained with reference solution (a), the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_4H_8O_2$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. When it is intended for use in preparing injectable dosage forms, the label states that it must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins.

Ethylcellulose

Cellulose ethyl ether

Ethylcellulose is an ethyl ether of cellulose.

Ethylcellulose contains not less than 44.0 per cent and not more than 51.0 per cent of ethoxy ($-OC_2H_5$) groups, calculated on the dried basis.

Category. Pharmaceutical aid.

Description. A white to light tan powder.

Identification

Dissolve 15 mg of the dried sample in 10 ml of dried *dichloromethane*. Grind 0.5 ml of the solution to dryness with 0.3 g of *potassium bromide*. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethylcellulose IPRS* or with the reference spectrum of ethylcellulose.

Tests

pH (2.4.24). 5.5 to 8.0, determined in a solution prepared in the following manner. Stir 1.0 g with 50 ml of *carbon dioxide-free water* previously heated to 90°, then cool and dilute with sufficient *carbon dioxide-free water* to produce 100 ml.

Apparent viscosity. 90.0 to 110.0 per cent of that stated on the label for viscosity types of 10 mPa s or more; 80.0 to 120.0 per cent of that stated on the label for viscosity types of 6 to 10 millipascal seconds; 75.0 to 140.0 per cent of that stated on the label for viscosity types of 6 millipascal seconds or less, determined by the following method. Weigh accurately about 5.0 g, calculated on the dried basis and dissolve in 95.0 ± 0.05 g of a mixture of 80 parts of *toluene* and 20 parts of *ethanol* by weight. For ethylcellulose containing less than 46.5 per cent of ethoxy groups use a mixture of 60 parts of *toluene* and 40 parts of *ethanol*. Determine the viscosity at 25° by Method A (2.4.28).

Arsenic (2.3.10). Mix 3.30 g with 5 ml of *sulphuric acid AsT*, add a few glass beads and digest in a fumehood, preferably on a hot plate at a temperature not exceeding 120°, until charring begins. (Additional acid may be necessary to wet some samples completely but the total volume added should not exceed 10 ml). Cautiously add, dropwise, *hydrogen peroxide solution (30 per cent)* allowing the reaction to subside and again heating between additions of drops. Add the first few drops very slowly with sufficient mixing to prevent a rapid reaction. Discontinue heating if foaming becomes excessive. When the reaction has abated, heat cautiously, rotating the flask occasionally to prevent the sample from caking on glass exposed to the heating unit. *(NOTE-Maintain oxidising conditions at all times during the digestion by adding small quantities of the hydrogen peroxide solution whenever the mixture turns brown or darkens).* Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the heating unit until fumes of sulphur trioxide are copiously evolved and the solution becomes colourless or retains only a light straw colour. Cool, add cautiously 10 ml of *water*, mix, and again evaporate till strong fuming, repeating this procedure to remove any trace of hydrogen peroxide. Cool, add cautiously 10 ml of *water*, wash the sides of the flask with a few ml of *water*, and dilute with *water* to 35 ml. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. To 10 ml of *o-xylene* add 0.5 ml of *octane* and dilute to 100.0 ml with *o-xylene.*

Test solution. To 30 mg (dried substance), add 60 mg of *adipic acid* in a 5 ml pressure–tight reaction vial equipped with a

pressure-tight membrane stopper coated with *polytetrafluoroethylene* and secured with an aluminium crimped cap or another sealing system providing a sufficient air-tightness. Add 2.0 ml of the internal standard solution and 1.0 ml of *hydriodic acid* and close immediately. Accurately weigh the vial (total mass before heating), do not mix the contents of the vial by hand before heating. Place the vial in an oven or heat in a suitable heater, with continuous mechanical agitation, maintaining the internal temperature of the vial at 115± 2° for 70 minutes. Allow to cool and accurately weigh the vial (total mass after heating). If the difference between the total mass before heating and the total mass after heating is more than 10 mg, prepare a new test solution. After phase separation, pierce through the septum of the vial with a cooled syringe and withdraw a sufficient volume of the upper layer as the test solution.

Reference solution. Place 60 mg of *adipic acid* and 2.0 ml of the internal standard solution in another 5 ml reaction vial, add 1.0 ml of *hydriodic acid* and close immediately. Accurately weigh the vial then inject 25 µl of *iodoethane* through the septum into the vial, weigh again accurately and mix. After phase separation, pierce through the septum of the vial with a cooled syringe and withdraw a sufficient volume of the upper layer as the reference solution.

Chromatographic system

- a fused silica column 30 m × 0.53 mm, packed with poly (dimethyl) siloxane (film thickness 3 µm),
- column temperature:

time (min.)	temperature (°)
0-3	50
3-8	50→100
8-12	100→250
12-20	250

- inlet port at 250° and detector at 280°,
- flow rate: 4.2 ml per minute using helium as carrier gas,
- flame ionization detector,
- split ratio: 1:40.

The relative retention time with reference to octane (retention time about 10 minutes) iodoethane about 0.6.

Inject 1µl of the reference solution. The test is not valid unless the resolution between the peaks due to iodoethane and octane is not less than 5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and internal standard solution.

Calculate the response factor of iodoethane using the following expression.

$$\frac{A_1 \times W_1 \times C}{A_2 \times 100}$$

where, A_1 = area of the peak due to the internal standard in the chromatogram obtained with the reference solution,

A_2 = area of the peak due to iodoethane in the chromatogram obtained with the reference solution,

W_1 = mass of iodoethane in the reference solution in mg,

C = percentage content of iodoethane.

Inject the reference solution, internal standard solution and the test solution.

Calculate the percentage content m/m of ethoxy groups using the following expression.

$$\frac{A_4 \times R \times M_1 \times 100}{A_3 \times W_2 \times M_2}$$

where, A_3 = area of the peak due to the internal standard in the chromatogram obtained with the test solution,

A_4 = area of the peak due to iodoethane in the chromatogram obtained with the test solution,

R = reponse factor,

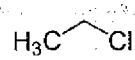
M_1 = molar mass of the ethoxygroups (45.1),

M_2 = molar mass of iodoethane (156.0),

W_2 = mass of the sample (dried substance) in the test solution, in mg.

Labelling. The label states the apparent viscosity in mPa s of a 2.0 per cent w/v solution and its ethoxy content.

Ethyl Chloride



C_2H_5Cl Mol. Wt. 64.5

Ethyl Chloride is chloroethane.

Category. Anaesthetic.

Description. Gaseous at ambient temperatures and pressures but is generally compressed to a colourless, mobile, flammable and very volatile liquid.

Identification

A. Burns with a luminous flame with the production of hydrogen chloride.

B. Hydrolyse a few ml with *5 M sodium hydroxide*; the resulting solution gives the reactions of chlorides (2.3.1), and after the addition of *iodine solution* and warming, crystals of iodoform are produced.

Tests

Acidity or alkalinity. Shake 10 ml with 10 ml of ice-cold *water* and allow the ethyl chloride to evaporate at room temperature; the residual liquid (liquid A) is neutral to *litmus solution*.

Ionisable chlorides. 5 ml of liquid A gives no turbidity with *silver nitrate solution*.

Ethanol. Warm 5 ml of liquid A with *iodine solution* and *sodium carbonate*; no iodoform is produced.

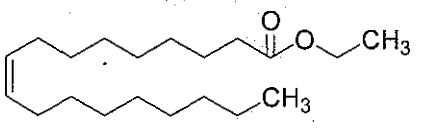
Distillation range. Into a dry 100-ml measuring cylinder insert a stopper carrying a short exit tube not less than 6 mm in internal diameter and an accurately standardised short-bulb thermometer covering the range –20° to +30° and graduated in tenths of a degree. Cover the bulb of the thermometer with a piece of very fine muslin, free from grease and sizing materials, so that one end hangs down about 10 mm below the bulb. Cool the cylinder in ice-water, transfer to it 100 ml of the sample, previously cooled in ice-water, insert the stopper and adjust the thermometer so that the end of the muslin dips into the liquid and the bulb is above the surface. Replace the ice-water with *water* at 24° to 26° and observe the temperature when 5 ml of sample has evaporated and again when 5 ml remains. Continually lower the thermometer to maintain its position relative to the liquid surface throughout the test. Correct the observed temperature by adding 0.26° for every kPa that the barometric pressure is below 101.3 kPa or by subtracting 0.26° for every kPa above. The corrected temperature is not lower than 12.0° and not higher than 12.5°.

Other organic compounds. On evaporation, no foreign odour is detectable at any stage.

Non-volatile matter. Not more than 0.01 per cent w/w, when evaporated and dried at 105°.

Storage. Store protected from light in a refrigerator (2° to 8°).

Ethyl Oleate



$C_{20}H_{38}O_2$ Mol. Wt. 310.5

Ethyl Oleate consists of the ethyl esters of (*Z*)-oleic and related acids.

Ethyl Oleate contains not less than 100.0 per cent w/w and not more than 105.0 per cent w/w of the ethyl esters of (*Z*)-oleic and related acids, calculated as $C_{20}H_{38}O_2$.

Category. Pharmaceutical aid.

Description. A clear pale yellow or colourless liquid.

Tests

Weight per ml (2.4.29). 0.869 g to 0.874 g, determined at 20°.

Acid value (2.3.23). Not more than 0.5.

Peroxides. Dissolve 5.0 g in 15 ml of *chloroform*, add 20 ml of *glacial acetic acid* and 0.5 ml of a saturated solution of *potassium iodide*, mix and allow to stand for exactly 1 minute in the dark. Add 30 ml of *water* and titrate with *0.01 M sodium thiosulphate* using *starch solution* as indicator; not more than 2.5 ml of *0.01 M sodium thiosulphate* is required.

Iodine value (2.3.28). 75 to 85.

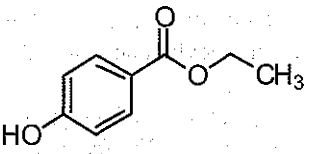
Assay. Boil a suitable volume of *ethanol (95 per cent)* to expel carbon dioxide and neutralise it to *phenolphthalein solution*. Weigh 2.0 g of the substance under examination, dissolve in 5 ml of the neutralised ethanol contained in a hard-glass flask and neutralise the free acid in the solution with *0.1 M ethanolic potassium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Add 25.0 ml of *0.5 M ethanolic potassium hydroxide* and boil under a reflux condenser on a water-bath for 1 hour and continue boiling for 2 hours over a flame. Add 20 ml of *water* and titrate the excess of alkali with *0.5 M hydrochloric acid* using a further 0.2 ml of *phenolphthalein solution* as indicator. Repeat the operation without the substance under examination. The difference between the titres represents the alkali required to saponify the substance under examination.

1 ml of *0.5 M ethanolic potassium hydroxide* is equivalent to 0.1553 g of $C_{20}H_{38}O_2$.

Storage. Store protected from light in small, well-filled and well-closed containers under an atmosphere of nitrogen.

Ethylparaben

Ethyl hydroxybenzoate; Ethyl parahydroxybenzoate.



$C_9H_{10}O_3$ Mol. Wt. 166.2

Ethylparaben is ethyl 4-hydroxybenzoate.

Ethylparaben contains not less than 98.0 per cent and not more than 102.0 per cent of $C_9H_{10}O_3$.

Category. Pharmaceutical aid.

Description. A white or almost white, crystalline powder or colourless crystals.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethylparaben IPRS* or with the reference spectrum of ethylparaben.

Tests

Solution A. A 10.0 per cent w/v solution in *ethanol (95 per cent)*.

Appearance of solution. Solution A is clear (2.3.1) and not more intensely coloured than reference solution BYS6 (2.3.1).

Acidity. To 2 ml of solution A, add 3 ml of *ethanol (95 per cent)*, 5 ml of *carbon dioxide- free water* and 0.1 ml of *bromocresol green solution*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 2.5 ml of *methanol* and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.0005 per cent w/v each of ethylparaben impurity A, ethylparaben impurity B and the substance under examination in the mobile phase.

Reference solution (b). Dissolve 50 mg of *ethylparaben IPRS* in 2.5 ml of *methanol* and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of 0.68 per cent w/v of *potassium dihydrogen phosphate* and 65 volumes of *methanol*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Ethylparaben impurity A[1]	0.5	1.4
Ethylparaben (Retention time: about 3 minutes)	1.0	—
Ethylparaben impurity B[2]	0.8	—

[1]4-hydroxybenzoic acid,

[2]methyl parahydroxybenzoate.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to ethylparaben impurity B and ethylparaben is not less than 2.0.

Inject reference solution (c) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. The area of any peak corresponding to ethylparaben impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

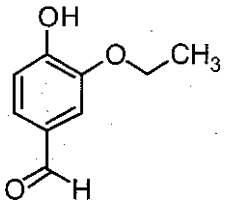
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14), as described under test for related substances with the following modification.

Inject reference solution (b) and the test solution.

Calculate the content of $C_9H_{10}O_3$.

Ethyl Vanillin



$C_9H_{10}O_3$ Mol. Wt.166.2

Ethyl Vanillin is 3-Ethoxy-4-hydroxybenzaldehyde.

Ethyl Vanillin contains not less than 98.0 per cent and not more than 101.0 per cent of $C_9H_{10}O_3$, calculated on the dried basis.

Category. Pharmaceutical aid.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethyl vanillin IPRS* or with the reference spectrum of ethyl vanillin.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.0008 per cent w/v solution in *methanol* shows absorption maxima similar to that in the reference solution.

Tests

Melting range (2.4.21). 76° to 78°.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

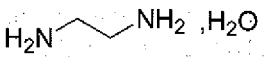
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1 g by drying over *phosphorous pentoxide* for 4 hours.

Assay. Dissolve 0.3 g, in 50 ml of *dimethylformamide* contained in a 125-ml conical flask. Add *thymol blue TS* and titrate with *0.1 M sodium methoxide*, using a magnetic stirrer and taking precautions against the absorption of atmospheric carbon dioxide. Carry out a blank titration.

1 ml of *0.1 M sodium methoxide* is equivalent to 0.01662 g of $C_9H_{10}O_3$.

Storage. Store protected from light and moisture.

Ethylenediamine Hydrate



$C_2H_8N_2,H_2O$ Mol. Wt. 78.1

Ethylenediamine Hydrate is ethane-1,2-diamine monohydrate.

Ethylenediamine Hydrate contains not less than 97.5 per cent w/w and not more than 101.5 per cent w/w of $C_2H_8N_2,H_2O$.

Category. Pharmaceutical aid (for Aminophylline Injection).

Description. A clear, colourless or slightly yellow liquid.

Identification

A. Dilute 1 ml to 6 ml with *water*. To 3 drops of the solution add 2 ml of a 1 per cent w/v solution of *copper sulphate* and shake; a purple-blue colour is produced.

B. It is strongly alkaline.

Tests

Ammonia and other bases. Weigh 1.5 ml and transfer with the aid of *ethanol (95 per cent)* to a small dish. Add, with stirring, 20 ml of *dilute hydrochloric acid*. Evaporate the solution to dryness on a water-bath, breaking up any cake formed with a glass rod, and dry at 105° for 1 hour.

1 g of residue is equivalent to 0.5872 g of $C_2H_8N_2,H_2O$.

Calculate the percentage of $C_2H_8N_2,H_2O$; the result is within 0.5 per cent of the percentage of ethylenediaminehydrate determined in the Assay.

Heavy metals (2.3.13). Evaporate 5.0 ml on a water-bath to dryness, add to the residue 1 ml of *hydrochloric acid* and 0.5 ml of *nitric acid* and evaporate to dryness. Dissolve the residue in 20 ml of warm *water*, cool, add sufficient *water* to produce 100 ml and mix. 20 ml of the resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

Iron (2.3.14). To the residue obtained in the test for Non-volatile matter add 1 ml of *hydrochloric acid* and 0.5 ml of *nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 20 ml of warm *water* and dilute with *water* to 100 ml. 40 ml of the solution complies with the limit test for iron (20 ppm).

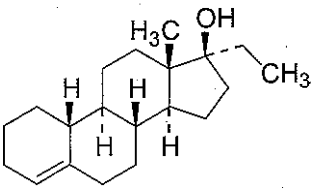
Non-volatile matter. Not more than 0.02 per cent w/v, determined on 5.0 ml by evaporating to dryness on a water-bath and drying at 105° for 1 hour.

Assay. Weigh 1.0 g, dissolve in 75 ml of *water* and titrate with *1 M hydrochloric acid* using *bromophenol blue solution* as indicator until a yellow colour is produced. Carry out a blank titration.

1 ml of *1 M hydrochloric acid* is equivalent to 0.03906 g of $C_2H_8N_2,H_2O$.

Storage. Store protected from light.

Ethyloestrenol



$C_{20}H_{32}O$ Mol. Wt. 288.5

Ethyloestrenol is 17α-ethylestr-4-en-17β-ol containing a variable amount of methanol of crystallisation.

Ethyloestrenol contains not less than 95.0 per cent and not more than 103.0 per cent of $C_{20}H_{32}O$, calculated on the anhydrous and methanol-free basis.

Category. Anabolic steroid.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethyloestrenol IPRS* or with the reference spectrum of ethyloestrenol.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *heptane* and 20 volumes of *acetone*.

Solvent mixture. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml in the solvent mixture.

Reference solution (a). A 0.25 per cent w/v solution of *ethyloestrenol IPRS* in the solvent mixture.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat it at 105° for 10 minutes, spray with *ethanolic sulphuric acid (20 per cent)* and heat at 105° for a further 10 minutes. Allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Specific optical rotation (2.4.22). +29.0° to +33.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

17α-Ethyloestran-17β-ol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* containing 20 per cent w/v of *silver nitrate*.

Mobile phase. A mixture of 75 volumes of *toluene* and 25 volumes of *nonan-5-one*.

Solvent mixture. 9 volumes of *chloroform* and 1 volume of *methanol*.

Test solution. Dissolve 0.4 g of the substance under examination in 10 ml in the solvent mixture.

Reference solution. A 0.08 per cent w/v solution of *17α–ethyloestran-17β-ol IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 105° for 10 minutes, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for a further 10 minutes and allow to cool. Any spot corresponding to 17α–ethyloestran-17β-ol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Related substances. Carry out Identification test B but using 10 µl of the following solutions.

Test solution. A 1 per cent w/v solution of the substance under examination.

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination.

Reference solution (b). A 0.005 per cent w/v solution of the substance under examination.

Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Methanol. Not more than 4.0 per cent w/w, determined by the following method.

Determine by gas chromatography (2.4.13).

Test solution (a). A solution containing 10.0 per cent w/v of the substance under examination in *acetone*.

Test solution (b). A solution containing 10.0 per cent w/v of the substance under examination and 0.4 per cent v/v of *ethanol* (internal standard) in *acetone*.

Reference solution. A solution containing 0.4 per cent v/v of *methanol* and 0.4 per cent v/v of the internal standard in *acetone*.

Chromatographic system

- a glass column 2.0 m x 0.4 mm, packed with porous polymer beads (100 to 120 mesh) (Such as Porapak Q),
- temperature:
  column.170°,
  inlet port and detector. 240°,
- flame ionisation detector,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of the reference solution, test solution (a) and (b).

Calculate the percentage w/w of methanol, assuming its weight per ml at 20° to be 0.792 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 5.0 g.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). A solution containing 0.2 per cent w/v of the substance under examination in *chloroform*.

Test solution (b). A solution containing 0.2 per cent w/v of the substance under examination and 0.1 per cent w/v of *arachidic alcohol* (internal standard) in chloroform.

Reference solution. A 0.2 per cent w/v solution of *ethyloestrenol IPRS* in *chloroform*.

Chromatographic system
- a glass column 1.0 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (Such as OV-17),
- temperature:
  column.200°,
  inlet port and detector. 280°,
- flame ionisation detector,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of the reference solution, test solution (a) and (b).

Calculate the content of $C_{20}H_{32}O$.

Storage. Store protected from light in a refrigerator (2° to 8°).

Ethyloestrenol Tablets

Ethyloestrenol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethyloestrenol, $C_{20}H_{32}O$.

Usual strength. 2 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *heptane* and 20 volumes of *acetone*.

Solvent mixture. 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Extract a quantity of the powdered tablets containing 1 mg of Ethyloestrenol with *chloroform*, filter, evaporate the filtrate to dryness at room temperature at a pressure not exceeding 0.2 kPa and dissolve the residue in 0.4 ml with solvent mixture.

Reference solution (a). A 0.25 per cent w/v solution of *ethyloestrenol IPRS* in the same solvent mixture.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 2 µl of each solution. After development, dry the plate in air, heat it at 105° for 10 minutes, spray with *ethanolic sulphuric acid (20 per cent)* and heat at 105° for a further 10 minutes. Allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. In the Assay, the principal peak the chromatogram obtained with the test solution has the same retention time as that of the peak due to *ethyloestrenol IPRS* in the chromatogram obtained with the reference solution (a).

Tests

17α-Ethyloestran-17β-ol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G* containing 20 per cent w/v of *silver nitrate*.

Mobile phase. A mixture of 75 volumes of *toluene* and 25 volumes of *nonan-5-one*.

Solvent mixture. 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Dissolve 20 mg of the residue obtained in the test for Related substances in 0.5 ml in the solvent mixture.

Reference solution. A 0.08 per cent w/v solution of *17α-ethyloestran-17β-ol IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 105° for 10 minutes, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for a further 10 minutes and allow to cool. Any spot corresponding to 17α-ethyloestran-17β-ol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Related substances. Carry out Identification test A but using 10 µl of the following solutions.

Solvent mixture. 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Extract a quantity of the powdered tablets containing 40 mg of Ethyloestrenol with *chloroform*, filter, evaporate the filtrate to dryness and dissolve 10 mg of the residue in 1 ml with solvent mixture.

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with the same solvent mixture.

Reference solution (b). Dilute 1 volume of reference solution (a) to 2 volumes with the same solvent mixture.

Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. Complies with the test stated under Tablets.

Proceed as directed in the Assay using the following solutions.

Test solution. Powder one tablet, extract with 5 ml of *chloroform* in a centrifuge tube, centrifuge, evaporate 2.0 ml of the supernatant liquid in a current of nitrogen, dissolve the residue

in 2 ml of *acetone*, evaporate the solution to dryness on a water-bath and dissolve the residue in 0.4 ml of *chloroform*.

Reference solution (a). A solution containing 0.2 per cent w/v of *ethyloestranol IPRS* and 0.1 per cent w/v of *arachidic alcohol* (internal standard) in *chloroform*.

Reference solution (b). Prepare in the same manner as the test solution on 2.0 ml of the supernatant liquid but by extracting with 2.0 ml of a 0.02 per cent w/v solution of *arachidic alcohol* in *acetone*.

Calculate the content of $C_{20}H_{32}O$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 8 mg of Ethyloestrenol with 20 ml of *acetone*, filter, evaporate the filtrate to dryness on a water-bath and dissolve the residue in 4.0 ml of *chloroform*.

Reference solution (a). A solution containing 0.2 per cent w/v of *ethyloestrenol IPRS* and 0.1 per cent w/v of *arachidic alcohol* (internal standard) in *chloroform*.

Reference solution (b). Prepared in a similar manner as solution (1) but extracting with 20 ml of a 0.02 per cent w/v solution of *arachidic alcohol* in *acetone*.

Chromatographic system

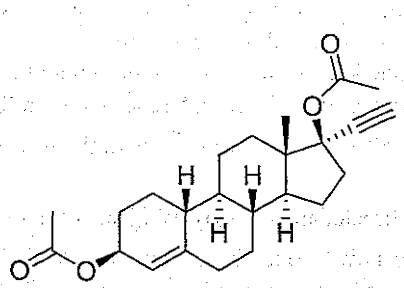
- a glass column 1.0 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (Such as OV-17),
- temperature:
  column. 200°,
  inlet port and detector. 280°,
- flame ionisation detector,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of reference solution (a), (b) and the test solution.

Calculate the content of $C_{20}H_{32}O$ in the tablets.

Storage. Store protected from light in a refrigerator (2° to 8°).

Ethynodiol Diacetate



$C_{24}H_{32}O_4$ Mol. Wt. 384.5

Ethynodiol Diacetate is 19-Norpregn-4-en-20-yne-3,17-diol, diacetate, (3β,17α).

Ethynodiol Diacetate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{24}H_{32}O_4$.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethynodiol diacetate IPRS* or with the reference spectrum of ethynodiol diacetate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Optical rotation (2.4.22). -76.0° to -70.0°, determined on 1.0 per cent w/v solution in *chloroform*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.25 g of the substance under examination in 50 ml of *acetonitrile*, with the aid of ultrasound and dilute to 100.0 ml with *water*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. Dissolve 125 mg of *ethynodiol diacetate IPRS* in 25 ml of *acetonitrile*, with the aid of ultrasound and dilute to 50.0 ml with *water*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with phenyl group (5 µm) (Such as Adsorbosphere Phenyl),
- column temperature: 40°,
- mobile phase: a mixture of 59 volumes of *water* and 41 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 20 µl.

Name	Relative retention time
α-Ethynodiol diacetate	0.87
Ethynodiol diacetate (Retention time: about 18 minute)	1.0

Inject the reference solution. The test is not valid unless the tailing factor is not less than 0.75 and not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the test solution. The area of any peak corresponding to α-ethynodiol diacetate is not more than 1.5 per cent, the

area of any other secondary peak is not more than 0.5 per cent and the sum of the areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization.

Limit of Conjugated Diene. The absorbance of a 0.05 per cent w/v solution in *methanol* at the maximum at about 236 nm (2.4.7) is not more than 0.50.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{32}O_4$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Ethynodiol Diacetate and Ethinyl Estradiol Tablets

Ethynodiol Diacetate and Ethinyl Estradiol Tablets contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of ethynodiol diacetate, $C_{24}H_{32}O_4$ and not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ethinyl estradiol, $C_{20}H_{24}O_2$.

Usual strength. Ethynodiol Diacetate, 1mg and Ethinyl Estradiol, 0.05 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with the reference solution.

Tests

Disintegration (2.5.1). Not more than 15 minutes, perform the test without discs.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4. 14), as described under Assay, with the following modifications.

Test solution. Disperse one intact tablet in 10 ml of the mobile phase, with the aid of ultrasound and dilute to 25.0 ml with the mobile phase.

Inject the reference solution and the test solution.

Calculate the contents of $C_{24}H_{32}O_4$ and $C_{20}H_{24}O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse 10 intact tablets in 200 ml of the mobile phase, with the aid of ultrasound and dilute to 250.0 ml with the mobile phase.

Reference solution. A solution containing 0.004 per cent w/v of *ethynodiol diacetate IPRS* and 0.0002 per cent w/v of *ethinyl estradiol IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups bonded to porous silica (5 µm) (Such as Zorbax CN),
- mobile phase: a mixture of 15 volumes of *methanol*, 35 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

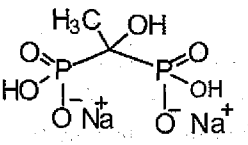
Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 1.5 for ethynodiol diacetate peak and the relative standard deviation for replicate injections is not more than 2.0 per cent for both the peaks.

Inject the reference solution and the test solution.

Calculate the contents of $C_{24}H_{32}O_4$ and $C_{20}H_{24}O_2$ in the tablets.

Storage. Store protected from moisture.

Etidronate Disodium



$C_2H_6Na_2O_7P_2$ Mol. Wt. 250.0

Etidronate Disodium is (1-Hydroxyethane-1,1-diyl)diphosphonic acid disodium salt.

Etidronate Disodium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_2H_6Na_2O_7P_2$, calculated on the anhydrous basis.

Category. Bone resorption inhibitor.

Description. A white or yellowish, hygroscopic powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etidronate disodium IPRS* or with the reference spectrum of etidronate disodium.

B. It gives reaction (A) of sodium salts (2.3.1).

Tests

pH (2.3.24). 4.2 to 5.2, determined on 1.0 per cent w/v solution in *carbon dioxide-free water.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in *water* and dilute to 10.0 ml with *water.*

Reference solution. To 2.0 ml of a 0.03 per cent w/v solution of etidronate impurity A *(orthophosphoric acid)* , add 2.0 ml of a 0.025 per cent w/v solution of etidronate impurity B *(phosphorous acid)* and dilute to 50.0 ml with *water.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with anion exachange resin bonded to silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 0.2 ml of *anhydrous formic acid* and 1000 ml of *water*, adjusted to pH 3.5 with 8.0 per cent w/v solution of *sodium hydroxide,*
- flow rate: 1 ml per minute,
- differential refractometer,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to etidronate impurity A and etidronate impurity B is not less than 2.5.

Inject the reference solution and the test solution. The area of peak corresponding to etidronate impurity A and B is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 5.0 per cent, determined on 1.0 ml of 1.0 per cent w/v solution in a mixture of equal volumes of *anhydrous acetic acid* and *formamide* using Method 3.

Assay. Dissolve 0.1 g in 2 ml of *formic acid* and dilute to 50 ml with *glacial acetic acid.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0125 g of $C_2H_6Na_2O_7P_2$.

Storage. Store protected from moisture.

Etidronate Tablets

Etidronate Tablets contain Etidronate Disodium.

Etidronate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of etidronate disodium, $C_2H_6Na_2O_7P_2$.

Usual strengths 200 mg; 400 mg.

Identification

A. To a quantity of the powdered tablets containing 1 g of Etidronate Disodium, add 10 ml of *water* and shake for 5 minutes, filter. Add 15 ml of *methanol* to precipitate the solution, mix and centrifuge. Discard the supernatant liquid and dry the residue at 105° for 1 hour. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etidronate disodium IPRS* or with the reference spectrum of etidronate disodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate, if necessary to produce a solution containing 0.022 per cent w/v of Etidronate Disodium.

Reference solution. A 0.022 per cent w/v solution of *etidronate disodium IPRS* in *water.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with anion exchange resin (10 µm),
- mobile phase: a 0.0454 per cent v/v solution of *nitric acid* in *water,*
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 200 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_2H_6Na_2O_7P_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_2H_6Na_2O_7P_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 20 mg of Etidronate Disodium in *water* with the aid of ultrasound and dilute to 10.0 ml with *water.*

Reference solution. To 4 volumes of a 0.03 per cent w/v solution of *orthophosphoric acid,* add 4 volumes of a 0.025 per cent w/v solution of *phosphorous acid* and dilute to 50 volumes with *water.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with anion exchange resin (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 0.2 volumes of *anhydrous formic acid* and 1000 volumes of *water*, adjusted to pH 3.5 with 8.0 per cent w/v solution of *sodium hydroxide*,
- flow rate: 1 ml per minute,
- differential refractometer,
- injection volume: 100 µl.

The retention times of phosphate peak is about 9.4 minutes and of phosphite peak is about 11.5 minutes.

Inject the reference solution. The test is not valid unless the resolution between the peaks corresponding to phosphate and phosphite is not less than 2.5.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to phosphite is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (1.0 per cent). The area of any peak corresponding to phosphate is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 44 mg of Etidronate Disodium in 190 ml of *water* with the aid of ultrasound for 5 minutes and shake for 10 minutes. Dilute to 200 ml with *water*. Centrifuge for 10 minutes and filter.

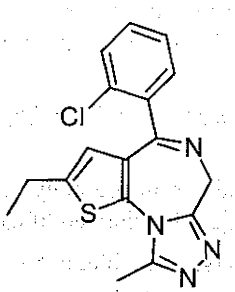
Reference solution. A 0.022 per cent w/v solution of *etidronate disodium IPRS* in *water*.

Use chromatographic system as described under Dissolution.

Inject the reference solution and the test solution.

Calculate the content of $C_2H_6Na_2O_7P_2$ in the tablets.

Etizolam



$C_{17}H_{15}ClN_4S$ Mol. Wt. 342.9

Etizolam is 4-(2-Chlorophenyl)-2-ethyl-9-methyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-α][1,4] diazepine.

Etizolam contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{17}H_{15}ClN_4S$, calculated on the dried basis.

Category. Antianxiety.

Description. A white to pale yellowish, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etizolam IPRS* or with the reference spectrum of etizolam.

B. When examined in the range from 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maxima at the same wavelength as that of *etizolam IPRS* prepared in the same manner.

C. Melting point (2.4.21). 146° to 149°.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in *acetonitrile* and dilute to 100.0 ml with *acetonitrile*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

Reference solution (b). A solution containing 0.008 per cent w/v each of *etizolam IPRS* and *ethyl parahydroxybenzoate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature: 35°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *dilute orthophosphoric acid* and 45 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to ethyl parahydroxybenzoate and etizolam is not less than 3.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. Run the chromatogram 5 times the retention time of the principal peak, the area of any secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent determined on 1 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.3 g in 70 ml of a mixture of 7 volumes of *acetic anhydride* and 3 volumes of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01714 g of $C_{17}H_{15}ClN_4S$.

Storage. Store protected from light and moisture.

Etizolam Tablets

Etizolam Tablets contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of etizolam, $C_{17}H_{15}ClN_4S$.

Usual strengths. 0.5 mg; 1 mg.

Identification

A. Shake a quantity of the powdered tablets containing 5 mg of etizolam, add 10 ml of *methanol* and filter. Evaporate the filtrate to dryness and dissolve the residue in 2 ml of *sulphuric acid*. The solution gives, a yellowish-green fluorescence, when examined under ultraviolet light at 365 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate with the dissolution medium to obtain a solution containing 0.000028 per cent w/v of Etizolam. Dilute 5.0 ml of the solution to 10.0 ml with *acetonitrile*.

Reference solution. Dissolve 28 mg of *etizolam IPRS* in 50 ml of *methanol* and dilute with the dissolution medium to obtain a solution containing 0.000028 per cent w/v of etizolam. To 2.0 ml of the solution, add 2.0 ml of *acetonitrile*.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *water* and *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 243 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{15}ClN_4S$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{17}H_{15}ClN_4S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 4 mg of Etizolam in 5 ml of the mobile phase, with the aid of ultrasound and dilute to 10.0 ml with the mobile phase and filter.

Reference solution. A 0.0004 per cent w/v solution of *etizolam IPRS* in the mobile phase.

Use chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 4500, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtain with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtain with the reference solution (2.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in 5 ml of *water* and dilute with *methanol* (*90 per cent*) to obtain a solution containing 0.001 per cent w/v of Etizolam.

Calculate the content of $C_{17}H_{15}ClN_4S$ in the tablet.

Other tests. Complies with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 10 mg of Etizolam in 10 ml of *water* and dilute to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with *methanol (90 per cent)*.

Reference solution. A 0.001 per cent w/v solution of *etizolam IPRS* in *methanol (90 per cent)*.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *dilute orthophosphoric acid* and 45 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

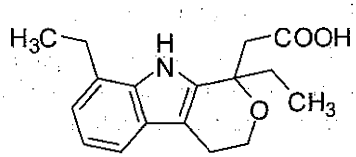
Inject the reference solution.The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{15}ClN_4S$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Etodolac



$C_{17}H_{21}NO_3$ Mol. Wt. 287.4

Etodolac is 1,8-diethyl-1,3,4,9-tetrahydropyrano [3,4-*b*]indole-1-acetic acid.

Etodolac contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{21}NO_3$, calculated on the anhydrous basis.

Category. Analgesic; antiinflammatory.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etodolac IPRS* or with the reference spectrum of etodolac.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 0.5 volume of *glacial acetic acid*, 30 volumes of *anhydrous ethanol* and 70 volumes of *toluene*.

Test solution. Dissolve 10 mg of the substance under examination in 10 ml of *acetone*.

Reference solution. A 0.1 per cent w/v solution of *etodolac IPRS* in *acetone*.

Impregnate the TLC plate by heating at 105° for 1 hour. Place the plate in an unsaturated chamber containing a mixture of 20 volumes of a 2.5 per cent w/v solution of *ascorbic acid* and 80 volumes of *methanol*. Allow the solution to rise 1 cm above the line of application on the plate, remove the plate and allow it to dry for 30 minutes.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm of the plate. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Melting point (2.4.21). 144° to 150°.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.02 g of the substance under examination in *acetonitrile* and dilute to 50.0 ml with *acetonitrile*.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with *acetonitrile*. Dilute 1.0 ml of the solution to 20.0 ml with *acetonitrile*.

Reference solution (b). Dissolve 4 mg of *2-(7-ethyl-1H-indol-3-yl)ethanol IPRS (etodolac impurity H)* in 10.0 ml of the test solution. Dilute 0.5 ml of the solution to 50.0 ml with *acetonitrile*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with endcapped butylsilane bonded to porous silica (3.5 µm),
- mobile phase: A. 0.077 per cent w/v solution of *ammonium acetate*,

B. a mixture of 10 volumes of mobile phase A and 90 volumes of *acetonitrile*,

- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
25	50	50
42	50	50
48	80	20

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to etodolac and etodolac impurity H is not less than 5.0. The relative retention time with reference to etodolac for 8-desethyl etodolac (etodolac impurity A) is about 0.68, for 8-methyl etodolac (etodolac impurity B) is about 0.83, for 1-methyl etodolac (etodolac impurity C) is about 0.85, for 2-(7-ethyl-1*H*-indol-3-yl)ethanol (etodolac impurity H) is about 1.09, for 8-isopropyl etodolac (etodolac impurity D) is about 1.17, for 1-propyl etodolac (etodolac impurity G) is about 1.19, for 8-propyl etodolac (etodolac impurity E) is about 1.2, for 1-isopropyl etodolac (etodolac impurity F) is about 1.22, for etodolac dimer (etodolac impurity I) is about 1.5 and for etodolac methyl ester (etodolac impurity K) is about 2.37.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak due to etodolac impurity C is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the area of each secondary peak corresponding to etodolac impurity A, B, D, E, F, G, H, I, K is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent); the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of all other secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Chlorides. Dissolve 1.0 g of the substance under examination in 60 ml of *methanol*, add 10 ml of *water* and 20 ml of *dilute nitric acid*. Titrate with *0.01 M silver nitrate*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.01 M silver nitrate* is equivalent to 0.0003545 g of Cl (300 ppm).

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh 0.25 g and dissolve in 60 ml of *methanol*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02874 g of $C_{17}H_{21}NO_3$.

Etodolac Capsules

Etodolac Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of etodolac, $C_{17}H_{21}NO_3$.

Usual strengths. 200 mg; 300 mg.

Identification

To a quantity of the contents of the capsules containing about 0.1 g of Etodolac, add 4 ml of *0.01 M hydrochloric acid* and mix with the aid of ultrasound for 5 minutes, shaking occasionally, centrifuge for 10 minutes, discard the supernatant liquid and wash the residue with 4 ml of *water*. Shake to disperse, centrifuge for 10 minutes and discard the supernatant liquid. Add 4 ml of *0.01 M sodium hydroxide* to the residue and mix with the aid of ultrasound for 5 minutes, shaking occasionally and centrifuge for 10 minutes. Transfer the supernatant liquid to a second centrifuge tube, add about 1 ml of *0.1 M hydrochloric acid*, the pH of the supernatant liquid should be 2 or less. Centrifuge for 10 minutes, discard the supernatant liquid and wash the residue with 4 ml of *water*, shake to disperse and centrifuge for 10 minutes. Discard the supernatant liquid and dry the residue at 105° for 1 hour. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etodolac IPRS* or with the reference spectrum of etodolac.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of *phosphate buffer pH 7.5*,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary with dissolution medium at 278 nm (2.4.7). Calculate the content of etodolac, $C_{17}H_{21}NO_3$ in the medium from the absorbances obtained from a solution of known concentration of *etodolac IPRS*.

Q. Not less than 70 per cent of the stated amount of $C_{17}H_{21}NO_3$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 0.5 volumes of *glacial acetic acid*, 30 volumes of *absolute ethanol* and 70 volumes of *toluene*.

Test solution. Shake a quantity of the contents of capsules containing 0.2 g of Etodolac with 20.0 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with *acetone*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 2.0 ml with *acetone*.

Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Etodolac acid dimer. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 3 volumes of *glacial acetic acid*, 17 volumes of *1,4-dioxan* and 60 volumes of *toluene*.

Test solution. Shake a quantity of the contents of capsules containing 0.6 g of Etodolac with 20.0 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter.

Reference solution. A 0.003 per cent w/v solution of *etodolac acid dimer IPRS* in *acetone*.

Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the acid dimer is not more intense than the spot in the chromatogram obtained with reference solution (0.1 per cent).

Total methyl analogue impurities. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the contents of capsules containing 0.1 g of Etodolac with 40 ml of *methanol*, mix with the aid of ultrasound for 5 minutes, filter and dilute 10.0 ml of the filtrate to 25.0 ml with *water*.

Reference solution. Dilute 1.0 ml of a solution containing 0.025 per cent w/v each of *etodolac 1-methyl analogue IPRS* and *etodolac 8-methyl analogue IPRS* in *methanol* to 50.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 13 volumes of *acetonitrile*, 19 volumes of *methanol* and 68 volumes of a 1.74 per cent w/v solution of *dipotassium hydrogen phosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to etodolac 8-methyl analogue and etodolac 1-methyl analogue is not less than 0.75.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak.

Calculate the content of etodolac 1-methyl analogue and etodolac 8-methyl analogue in etodolac. Total content is not more than 1.0 per cent.

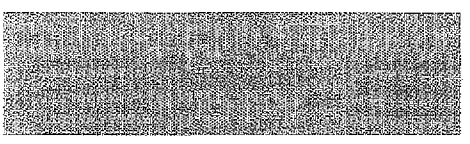
Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the mixed contents of 20 capsules containing 50 mg of Etodolac with about 70 ml of *0.1 M sodium hydroxide* for 30 minutes, dilute to 100.0 ml with *0.1 M sodium hydroxide*, mix and filter through a glass-fibre filter. Dilute 2.0 ml of the filtrate to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 2.0 ml of a 0.05 per cent w/v solution of *etodolac IPRS* in *0.1 M sodium hydroxide* to 100.0 ml with the mobile phase.

Reference solution (b). Add 2.0 ml of a 0.05 per cent w/v solution of *etodolac 1-methyl analogue IPRS* in *0.1 M sodium hydroxide* to 2.0 ml of a 0.05 per cent w/v solution of *etodolac IPRS* in *0.1 M sodium hydroxide* and dilute to 100 .0 ml with the mobile phase.



Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *phosphate buffer pH 4.75*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to etodolac and etodolac 1-methyl analogue is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{21}NO_3$ in the capsules.

Etodolac Prolonged-release Tablets

Etodolac Sustained-release Tablets; Etodolac Extended-release Tablets

Etodolac Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Etodolac Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of etodolac, $C_{17}H_{21}NO_3$.

Usual strengths. 200 mg; 600 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 600 mg of Etodolac in 100.0 ml of *acetonitrile* and shake by mechanical means for 40 minutes and dilute to 200.0 ml with the *acetonitrile*, mix and filter. Dilute 2.0 ml of the solution to 10.0 ml with *acetonitrile*.

Reference solution. A 0.048 per cent w/v solution of *etodolac IPRS* and 0.005 per cent w/v solution of *etodolac related compound A IPRS (±-8-ethyl-1,3,4,9- tetrahydropyrano [3,4-b]-indole-1-acetic acid)* in *acetonitrile*.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octylsilance bonded to porous silica (5 µm) and a guard column 4 cm x 4.0 mm, packed with the same column material,
- mobile phase: a mixture of 500 volumes of *acetonitrile*, 500 volumes of *water* and 0.25 volumes of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume: 10 µl.

The relative retention time with reference to etodolac for etodolac impurity A is about 0.8.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to etodolac impurity A and etodolac is not less than 2.5.

Inject the test solution. The area of any secondary peak is not more than 0.2 per cent and the sum of areas of all the secondary peaks is not more than 0.75 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14) as given under the test for Related substances using the following modification.

Reference solution. A 0.06 per cent w/v solution of *etodolac IPRS* in *acetonitrile*.

Inject the reference solution.The test is not valid unless the tailing factor is not more than 2.0 and relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{21}NO_3$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Etodolac Tablets

Etodolac Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of etodolac, $C_{17}H_{21}NO_3$.

Usual strengths. 200 mg; 300 mg; 400 mg.

Identification

Shake a quantity of the powdered tablets containing 0.5 g of etodolac with 30 ml of *hexane* for 5 minutes, centrifuge, discard the clear hexane layer and add about 40 ml of *ether* to the residue, shake for 5 minutes, centrifuge for 5 minutes, decant the ether layer and filter if necessary. Evaporate the solution

to dryness under nitrogen and add about 5 ml of *0.1 M hydrochloric acid* to the residue. Warm on a water-bath until the residue begins to crystallise and triturate with a glass rod to promote crystallisation. Cool the mixture in an ice bath, filter through a glass-fibre filter and dry the crystals at a pressure of 2 kPa at 60° for 1 hour, Complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etodolac IPRS* or with the reference spectrum of etodolac.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *phosphate buffer pH 7.5*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 278 nm (2.4.7). Calculate the content of etodolac, $C_{17}H_{21}NO_3$ in the medium from the absorbance obtained from a solution of known concentration of *etodolac IPRS*.

Q. Not less than 70 per cent of the stated amount of $C_{17}H_{21}NO_3$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 0.5 volumes of *glacial acetic acid*, 30 volumes of *absolute ethanol* and 70 volumes of *toluene*.

Test solution. Shake a quantity of the powdered tablets containing 0.2 g of Etodolac with 20.0 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with *acetone*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 2.0 ml with *acetone*.

Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.25 per cent).

Etodolac acid dimer. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 3 volumes of *glacial acetic acid*, 17 volumes of *1,4-dioxan* and 60 volumes of *toluene*.

Test solution. Dissolve a quantity of the powdered tablets containing about 0.6 g of Etodolac with 20.0 ml of *acetone*, mix with the aid of ultrasound for 5 minutes and filter.

Reference solution. A 0.003 per cent w/v solution of *etodolac acid dimer IPRS* in *acetone*.

Place the plate in an unlined tank containing a solution prepared by dissolving 0.5 g of L-*ascorbic acid* in 20 ml of *water* and adding 80 ml of *methanol*. Allow the solution to ascend 1 cm above the line of application on the plate, remove the plate and allow it to dry for at least 30 minutes.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to the acid dimer is not more intense than the spot in the chromatogram obtained with the reference solution (0.1 per cent).

Total methyl analogue impurities. Total content is not more than 1.0 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the powdered tablets containing 0.1 g of Etodolac with 40 ml of *methanol*, mix with the aid of ultrasound for 5 minutes and filter. Dilute 10.0 ml of the filtrate to 25.0 ml with *water*.

Reference solution. Dilute 1.0 ml of a solution containing 0.025 per cent w/v each of *etodolac 1-methyl analogue IPRS* and *etodolac 8-methyl analogue IPRS* in *methanol* to 50.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 13 volumes of *acetonitrile*, 19 volumes of *methanol* and 68 volumes of a 1.74 per cent w/v solution of *dipotassium hydrogen phosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to etodolac 8-methyl analogue and etodolac 1-methyl analogue is not less than 0.75.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak.

Calculate the content of etodolac 1-methyl analogue and etodolac 8-methyl analogue in etodolac.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Etodolac with 70 ml of *0.1 M sodium hydroxide* for 30 minutes, dilute to 100.0 ml with *0.1 M sodium hydroxide*, mix and filter through a glass-fibre filter. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 2.0 ml of a 0.05 per cent w/v solution of *etodolac IPRS* in *0.1 M sodium hydroxide* to 100.0 ml with the mobile phase.

Reference solution (b). Add 2.0 ml of a 0.05 per cent w/v solution of *etodolac 1-methyl analogue IPRS* in *0.1 M sodium hydroxide* to 2.0 ml of a 0.05 per cent w/v solution of *etodolac IPRS* in *0.1 M sodium hydroxide* and dilute to 100 .0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *phosphate buffer pH 4.75*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between peaks due to etodolac and etodolac 1-methyl analogue is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{17}H_{21}NO_3$ in the tablets.

Etophylline and Theophylline Prolonged-release Tablets

Etophylline and theophylline Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable.

Etophylline and Theophylline Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of etophylline, $C_9H_{12}N_4O_3$ and theophylline, $C_7H_8N_4O_2,H_2O$.

Usual strength. 115 mg Etophylline and 35 mg Theophylline.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 115 mg of Etophylline in 50 ml of *water*, with the aid of ultrasound and dilute to 200.0 ml with *water*. Stir this solution for 2 hours with magnetic stirrer, centrifuge at 3000 rpm for 5 minutes. Dilute 5.0 ml of the supernatant liquid to 25.0 ml with *water*, filter.

Reference solution. A solution containing 0.0115 per cent w/v of *etophylline IPRS* and 0.0035 per cent w/v of *theophylline IPRS* in *water*.

Chromatographic system

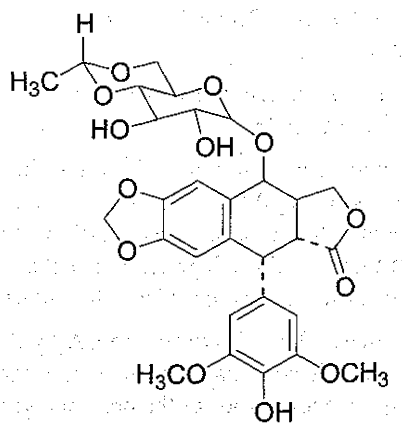
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of a buffer solution prepared by dissolving 2.72 g of *sodium acetate trihydrate* in 200 ml of *water*, add 10 ml of *glacial acetic acid* and dilute to 2000 ml with *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_{12}N_4O_3$ and $C_7H_8N_4O_2$ in the tablets

Etoposide



$C_{29}H_{32}O_{13}$ Mol. Wt. 588.6

Etoposide is 4′-demethylepipodophyllotoxin 9-[4,6-*O*-ethylidene-β-D-glucopyranoside].

Etoposide contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{29}H_{32}O_{13}$, calculated on the anhydrous basis.

CAUTION — Etoposide is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.

Category. Anticancer.

Description. A white or almost white crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etoposide IPRS*, or with the reference spectrum of etoposide.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Solvent mixture. 1 volume of *methanol* and 9 volumes of *dichloromethane*.

Mobile phase. A mixture of 1.5 volumes of *water*, 8 volumes of *glacial acetic acid*, 20 volumes of *acetone* and 100 volumes of *dichloromethane*.

Test solution. Dissolve 50 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution. A 0.5 per cent w/v solution of *etoposide IPRS* in the solvent mixture.

Apply to the plate 5µl of each solution as bands 10 mm by 3 mm. Allow the mobile phase to rise 17 cm. Dry the plate in warm air, spray it with a mixture of 1 volume of *sulphuric acid* and 9 volumes of *ethanol* and heat at 140° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve 5 mg in 5 ml of *glacial acetic acid* and add 0.05 ml of *ferric chloride solution*. Mix and cautiously add 2 ml of *sulphuric acid*. Avoid mixing the 2 layers. Allow to stand for about 30 minutes; a pink to reddish-brown ring develops at the interface and the upper layer is yellow.

Tests

Appearance of solution. A 3.0 per cent w/v solution in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane* is clear (2.4.1) and not more intensely coloured than reference solution YS6 or BYS6 (2.4.1).

Specific optical rotation (2.4.22). –114.0° to –106.0°, determined in a 0.5 per cent w/v solution in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution (a). Dissolve 40 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Test solution (b). Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b). To 10 ml of test solution (b), add 0.1 ml of a 4 per cent v/v solution of *glacial acetic acid* and 0.1 ml of *phenolphthalein solution*. Add *1 M sodium hydroxide* until the solution becomes faintly pink (about 0.15 ml). After 15 minutes, add 0.1 ml of a 4 per cent v/v solution of *glacial acetic acid*.

Reference solution (c) A 0.1 per cent w/v solution of *etoposide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 1 volume of *triethylamine*, 1 volume of *anhydrous formic acid* and 998 volumes of *water*,
  B. a mixture of 1 volume of *triethylamine*, 1 volume of *anhydrous formic acid* and 998 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
7	75	25
23	27	73
25	75	25

Name	Relative retention time	Correction factor
Etoposide impurity D[1]	0.4	—
Etoposide impurity E[2]	0.8	—
Etoposide (Retention time: about 5 minutes)	1.0	—
Etoposide impurity C[3]	1.1	—
Etoposide impurity B[4]	1.2	—
Etoposide impurity N[5]	3.1	—
Etoposide impurity O[6]	4.2	1.7

[1]lignan P,

[2]4'-desmethylepipodophyllotoxin,

[3]α-etoposide,

[4]*cis*-etoposide,

[5](5*R*,5a*R*,8a*R*,9*S*)-9-[[4,6-*O*-[(*R*)-ethylidene]-β-D-glucopyranosyl]oxy]-5-[4-[[(5*R*,5a*R*,8a*R*,9*S*)-5-(4-hydroxy-3,5-dimethoxyphenyl)-6-oxo-5,5a,6,8,8a,9-hexahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-9-yl]oxy]3,5-dimethzxoxyphenyl]-5,8,8a,9-tetrahydroisobenzofuro-[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one,

[6](5*R*,5a*R*,8a*R*,9*S*)-9-[[2,3-bis-*O*-(dichloroacetyl)-4,6-*O*-[(*S*)-ethylidene]-β-L-glucopyranosyl]oxy]-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8a,9-tetrahydroisobenzofuro[5,6-*f*][1,3]benzodioxol-6(5a*H*)-one.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to etoposide and to *cis*-etoposide is not less than 3.0. Ignore any peak due to phenolphthalein.

Inject reference solution (a) and test solution (a). The area of any peak corresponding to etoposide impurities B, C, D, E and N is not more than the twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any peak corresponding to etoposide impurity O is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.25 g.

Assay. Determine by liquid chromatography (2.4.14).

Follow the chromatographic procedure described under Related substances.

Inject reference solution (c). The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (c) and test solution (b).

Calculate the content of $C_{29}H_{32}O_{13}$.

Storage. Store protected from moisture.

Etoposide Capsules

Etoposide Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of etoposide, $C_{29}H_{32}O_{13}$.

CAUTION —Etoposide is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.

Usual strengths. 50 mg; 100 mg.

Identification

Add a quantity of the contents of the capsules containing 0.1 g of Etoposide to a separating funnel containing 100 ml of *water*, extract with two quantities, each of 20 ml, of *dichloromethane*, dry the combined organic extracts over *anhydrous sodium sulphate* and filter. Extract the filtrate with 30 ml of *water*, filter the dichloromethane layer through *anhydrous sodium sulphate* and evaporate to dryness at 25° to 35° under reduced pressure. Dissolve the oily residue in 5 ml of *water*, shake gently and allow to stand for 30 minutes. Filter through a sintered-glass funnel, wash the precipitate in the funnel with three quantities, each of 20 ml, of *water* and dry the precipitate in the funnel at 40° at a pressure of 2 kPa for 90 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etoposide IPRS*.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of a pH 4,5 buffer prepared by dissolving 2.99 g of *sodium acetate* and 14 ml of *2 M acetic acid* in 1000 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). A 0.005 per cent w/v solution of *etoposide IPRS* in the dissolution medium.

Reference solution (b). A solution containing 0.005 per cent w/v of *etoposide IPRS* and 0.00025 per cent w/v of *ethyl parahydroxybenzoate* in the dissolution medium.

Chromatographic system
- a stainless steel column 30 cm × 3.9 mm, packed with phenyl silica gel (10 µm) (Such as Bondapak phenyl),
- mobile phase: a mixture of 26 volumes of *acetonitrile* and 74 volumes of a 0.272 per cent w/v solution of *sodium acetate* adjusted to pH 4.0 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Q. Not less than 80 per cent of the stated amount of $C_{29}H_{32}O_{13}$.

***cis*-Etoposide**. Determine by liquid chromatography (2.4.14).

Test solution (a). Weigh a quantity of the mixed contents of 20 capsules containing about 0.5 g of Etoposide, dissolve in the mobile phase and dilute to 100.0 ml with the mobile phase; use immediately.

Test solution (b). Dilute 1.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution. A 0.5 per cent w/v solution of *etoposide IPRS* in a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volume of *triethylamine* and allow to stand for 40 minutes.

Use the chromatographic system described under Dissolution.

Inject the reference solution. The test is not valid unless the resolution between the principal peak and the peak immediately following the principal peak (*cis*-etoposide) is at least 1.0.

Inject test solution (a). The area of any peak corresponding to cis-etoposide is not more than the area of the peak in the chromatogram obtained with test solution (b) (2 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing about 40 mg of Etoposide dissolve in the mobile phase and dilute to 100.0 ml with the mobile phase; use immediately.

Reference solution (a). A 0.04 per cent w/v solution of *etoposide IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v of *etoposide IPRS* and 0.00025 per cent w/v of *ethyl parahydroxybenzoate* in the mobile phase.

Use the chromatographic system described under Dissolution.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 2.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{29}H_{32}O_{13}$ in the capsules.

Storage. Store protected from moisture at a temperature not exceeding 30°. The capsules should not be stored in a refrigerator.

Etoposide Injection

Etoposide Injection is a sterile material consisting of Etoposide Concentrate. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections in accordance with the manufacturer's instructions, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Infusions).

Usual strength. 20 mg per ml.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Etoposide Concentrate

Etoposide Concentrate is a sterile solution of Etoposide in a suitable ethanolic vehicle.

The concentrate complies with the requirements stated under Parenteral Preparations (Concentrated Solutions for Injection) and with the following requirements.

Etoposide Concentrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of etoposide, $C_{29}H_{32}O_{13}$.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 80 volumes of *dichloromethane*, 25 volumes of *acetone*, 2.5 volumes of *ethanol (95 per cent)* and 0.5 volume of *water*.

Test solution. Dilute a volume containing 20 mg of Etoposide to 25 ml with a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol*.

Reference solution. A 0.08 per cent w/v solution of *etoposide IPRS* in a mixture of 9 volumes of *dichloromethane* and 1 volume of *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in air, and examine under

ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 4.0, determined in a solution prepared by diluting a volume of the concentrate containing 0.1 g of Etoposide to 50 ml with *carbon dioxide-free water*.

***cis*-Etoposide**. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the concentrate containing 0.5 g of Etoposide to 100 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 50 ml with the mobile phase.

Reference solution (b). A 0.5 per cent w/v solution of *etoposide IPRS* in a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 0.1 volume of *triethylamine* and allow to stand for 40 minutes.

Use the chromatographic system described under Assay.

Inject reference solution (b). The test is not valid unless the resolution between the principal peak and the peak immediately after the principal peak (*cis*-etoposide) is not less than 1.0.

Inject reference solution (a) and the test solution. The area of any peak corresponding to *cis*-etoposide is not more than the area of the principal peak in the chromatogram obtained with reference solution (a).

Bacterial endotoxins (2.2.3). Dilute the injection in *water for injection* to obtain a concentration of 0.31 mg Etoposide activity per ml; it contains not more than 2.0 Endotoxin Units per mg of etoposide.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the concentrate containing about 40 mg of Etoposide to 100.0 ml with the mobile phase.

Reference solution. A 0.04 per cent w/v solution of *etoposide IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with phenyl silica gel (10 µm)(Such as µ Bondapak phenyl),
- mobile phase: a mixture of 26 volumes of *acetonitrile* and 74 volumes of a 0.272 per cent w/v solution of *sodium acetate* adjusted to pH 4.0 with *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

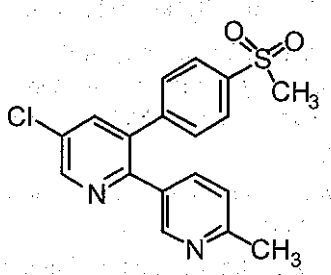
Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{32}O_{13}$ in the concentrate.

Storage. Store protected from light.

Labelling. The label states: (1) the directions for dilution of the contents; (2) that the diluted injection is to be given by intravenous injection; (3) that the concentrate should be protected from light.

Etoricoxib



$C_{18}H_{15}ClN_2O_2S$ Mol. Wt. 358.8

Etoricoxib is 5-Chloro-2-(6-methyl-3-pyridinyl)-3-(4-methylsulfonylphenyl)pyridine.

Etoricoxib contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{15}ClN_2O_2S$, calculated on the dried basis.

Category. Non steroidal anti-inflammatory drug.

Description. An off white to creamish coloured powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *etoricoxib IPRS* or with the reference spectrum of etoricoxib.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.0005 per cent w/v solution of *etoricoxib IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *0.05 M ammonium acetate* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The sum of the area of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 60° under vaccum for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *etoricoxib IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{15}ClN_2O_2S$.

Storage. Store protected from light and moisture.

Etoricoxib Tablets

Etoricoxib Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of etoricoxib, $C_{18}H_{15}ClN_2O_2S$.

Usual strengths. 60 mg; 90 mg; 120 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of buffer solution prepared by dissolving 1.38 g of *monobasic sodium phosphate* and 10 g of *sodium lauryl sulphate* in 1000 ml of *water*, adjusted to pH 7.0 with *1 M sodium hydroxide*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the dissolution medium, if necessary, at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{18}H_{15}ClN_2O_2S$ in the medium from the absorbance obtained from a solution of known concentration of *etoricoxib IPRS* in the dissolution medium in such manner to get similar concentration of the test solution.

Q. Not less than 70 per cent of the stated amount of $C_{18}H_{15}ClN_2O_2S$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of Etoricoxib with 50 ml of *methanol* with the aid of ultrasound and dilute to 100.0 ml with *methanol*. Dilute 1.0 ml of the solution to 10.0 ml with *methanol*.

Reference solution. A 0.005 per cent w/v solution of *etoricoxib IPRS* in *the methanol*.

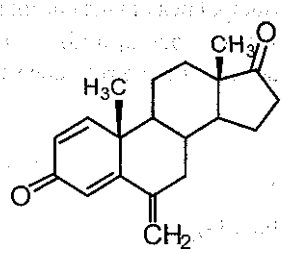
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *orthophosphoric acid* and 45 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{15}ClN_2O_2S$ in tablets.

Exemestane



$C_{20}H_{24}O_2$ Mol.Wt. 296.4

Exemestane is 6-methyleneandrosta-1, 4-diene-3, 17-dione.

Exemestane contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{20}H_{24}O_2$, calculated on the dried basis.

Category. Aromatase inhibitors.

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *exemestane IPRS* or with the reference spectrum of exemestane.

B. When examined in the range 220 to 300 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)*, shows absorption maxima at 246 nm the absorbance at the absorption maxima is between 0.467 to 0.495.

Tests

Melting range (2.4.21). 192° to 195°.

Specific optical rotation (2.4.22). +280° to +300°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in *acetonitrile* and dilute to 10.0 ml with *acetonitrile*.

Reference solution. A solution containing 0.00125 per cent w/v, each of, *exemestane IPRS, 1,4-andrastadione IPRS, 4-andrastadione IPRS* and *6- methyleneandrosta-4-ene-3,17-dione IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* previously adjusted to pH 4.5 with *orthophosphoric acid* and 40 volumes of *acetonitrile*.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Name	Relative retention time
1,4-Andrastadione	0.70
4-Andrastadione	0.95
Exemestane (Retention time: about 18 minute)	1.00
6- Methyleneandrosta-4-ene-3,17-dione	1.35

Inject the reference solution. The test is not valid unless the resolution between the peak due to 4-andrastadione and exemestane is not less than 1.5.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 1,4-andrastadione; 4-andrastadione and 6 - methyleneandrosta-4-ene-3,17-dione is not more than 0.6 times the area of the respective peak in the chromatogram obtained with the reference solution (0.3 per cent), the area of any other secondary peak is not more than 0.2 times the area of the exemestane peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all secondary peaks is not more than 2 times the area of the peaks corresponding to principal peak obtained with reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metal, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 25 mg of substance under examination *in ethanol (95 per cent)* and dilute to 25 ml with *ethanol (95 per cent)*. Dilute 1.0 ml of the solution to 100.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at 246 nm (2.4.7).

Calculate the content of $C_{20}H_{24}O_2$ taking 481 as the specific absorbance at 246 nm.

Storage. Store protected from moisture.

Exemestane Tablets

Exemestane Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of exemestane $C_{20}H_{24}O_2$.

Usual strength. 25mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of 0.5 per cent w/v *sodium lauryl sulphate*,

Speed and time. 100 rpm and 45 minutes

Withdraw a suitable volume of the medium and filter.

Measure the absorbance of the filtrate suitably diluted if necessary, with the dissolution medium at the maximum at about 246 nm (2.4.7). Calculate the content of $C_{20}H_{24}O_2$ in the medium from the absorbance obtained from a solution of *exemestane IPRS*, prepared by dissolving in minimum quantity of *ethanol (95 per cent)* and diluted suitably with the dissoluion medium to get similar concenration of the test soluion.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{24}O_2$.

Related substances. Determine by liquid chromatography (24.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powdered tablets containing 50 mg of exemestane in 30.0 ml of *acetonitrile* with the aid of ultrasound for 5 minutes and dilute to 50.0 ml with acetonitrile and filter and filter. Dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution. A solution containing 0.000125 per cent w/v, each of, *exemestane IPRS, 1,4-andrastadione IPRS, 4-andrastadione IPRS* and *6- methyleneandrosta-4-ene-3,17-dione IPRS* in *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphiric acid* in 1000 ml of *water* previously adjusted to pH 4.5 with *orthophosphoric acid* and 40 volumes of *acetonitrile.*
- flow: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Name	Relative retention time
1,4-Andrastadione	0.70
4-Andrastadione	0.95
Exemestane (Retention time: about 18 minute)	1.00
6-Methyleneandrosta-4-ene-3,17-dione	1.35

Inject the reference solution. The test is not valid unless the resolution between the peak due to 4-andrastadione and exemestane is not less than 1.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 4-andrastadione and 1,4-andrastadione is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent), the area of any peak corresponding to 6-methyleneandrosta-4-ene-3,17-dione is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent), the area of any other secondary peak is not more than 0.4 times the area of the exemestane peak in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of all the impurities is not more than 2.0 per cent.

Other tests. Comply with the test stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under test for Related substances with the following modifications.

Reference solution. A solution containing 0.025 per cent w/v solution of *exemestane IPRS* prepared by dissolving in minimum quantity of acetonitrile and further diluted with the mobile phase.

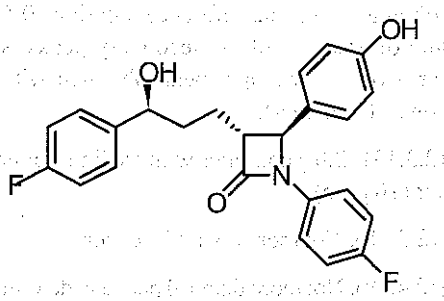
Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{24}O_2$ in the tablet.

Storage. Store protected from moisture.

Ezetimibe



$C_{24}H_{21}F_2NO_3$ Mol Wt. 409.4

Ezetimibe is (3*R*,4*S*)-1-(4-Fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)-2-azetidinone.

Ezetimibe contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{24}H_{21}F_2NO_3$, calculated on the dried basis.

Category. Antihyperlipidemic.

Description. A white to off white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ezetimibe IPRS* or with the reference spectrum of ezetimibe.

Tests

Specific optical rotation (2.4.22). −34.0° to −25.0°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of *acetonitrile*.

Reference solution. A 0.001 per cent w/v solution of *ezetimibe IPRS* in *acetonitrile*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 0.77 g of *ammonium acetate* in 1000 ml of *water* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of the *acetonitrile*.

Reference solution. A 0.02 per cent w/v solution of *ezetimibe IPRS* in *acetonitrile*.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{21}F_2NO_3$.

Storage. Store protected from light and moisture.

Ezetimibe Tablets

Ezetimibe Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ezetimibe, $C_{24}H_{21}F_2NO_3$.

Usual strength. 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 1.0 per cent w/v *sodium lauryl sulphate*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.025 per cent w/v solution of *ezetimibe IPRS* in the mobile phase. Further dilute 5.0 ml of the solution to 100.0 ml with the dissolution medium.

Use chromatographic system as described under Assay.

Calculate the content of $C_{24}H_{21}F_2NO_3$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{24}H_{21}F_2NO_3$.

Uniformity of content. Complies with the test stated under tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in 30 ml of the mobile phase, with the aid of ultrasound for 15 minutes and dilute to 50.0 ml with the mobile phase. Centrifuge and use the supernatant liquid.

Calculate the content of $C_{24}H_{21}F_2NO_3$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 25 mg of Ezetimibe with 15 ml of the mobile phase, with the aid of ultrasound 10 minutes and dilute to 25.0 ml with the mobile phase, centrifuge. Dilute 5.0 ml of the supernatant liquid to 25.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *ezetimibe IPRS* in the mobile phase.

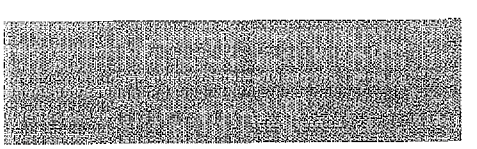
Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 0.77 g of *ammonium acetate* in 1000 ml of *water* and 50 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

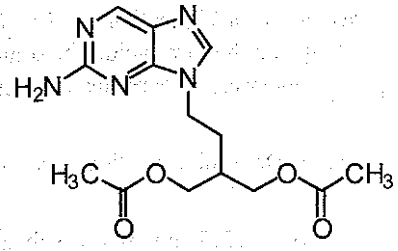
Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{21}F_2NO_3$ in the tablets.



F

Famciclovir



$C_{14}H_{19}N_5O_4$ Mol. Wt. 321.3

Famciclovir is 2-Acetoxymethyl-4-(2-amino-9*H*-purine-9-yl)butyl acetate

Famciclovir contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{19}N_5O_4$, calculated on anhydrous basis.

Category. Antiviral.

Description. A white to cream coloured powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *famciclovir IPRS* or with the reference spectrum of famciclovir.

B. When examined in the range from 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* exhibits absorption maxima at 222 nm, 243 nm and 309 nm (2.4.7).

Tests

pH (2.4.24). 6.0 to 7.5, determined on 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Use chromatographic system as described under Assay using detector wavelength at 221 nm.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent. The sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *famciclovir IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *0.02 M potassium dihydrogen orthophosphate* and 20 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{19}N_5O_4$.

Famciclovir Tablets

Famciclovir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of famciclovir, $C_{14}H_{19}N_5O_4$.

Usual strengths. 250 mg; 500 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *mixed phosphate buffer pH 6.8*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate if necessary with the dissolution medium and measure the absorbance at the maximum at about 305 nm (2.4.7). Calculate the content of $C_{14}H_{19}N_5O_4$ in the medium from the absorbance obtained from a solution of known concentration of *famciclovir IPRS* in the dissolution medium.

Q. Not less than 70 per cent of the stated amount $C_{14}H_{19}N_5O_4$.

Other tests. Comply with the tests stated in the Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Famciclovir in 30 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 50.0 ml with the mobile phase, filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *famciclovir IPRS* in the mobile phase.

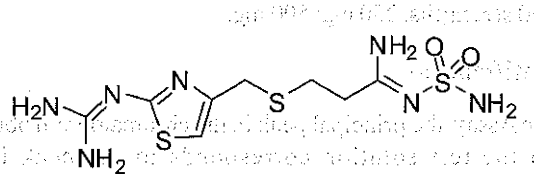
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of buffer solution prepared by dissolving 3.85 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 7.0 with *ammonia solution* and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at: 300 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{19}N_5O_4$ in the tablets.

Famotidine



$C_8H_{15}N_7O_2S_3$ Mol. Wt. 337.5

Famotidine is [1-amino-3-[[[2-(diaminomethylene)amino]-4-thiazolyl]methyl]thio]propylidene]sulphamide.

Famotidine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_8H_{15}N_7O_2S_3$, calculated on the dried basis.

Category. Antiulcer.

Description. A white or yellowish-white, crystalline powder or crystals.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *famotidine IPRS* or with the reference spectrum of famotidine.

Tests

Appearance of solution. Dissolve 0.2 g in a 5.0 per cent w/v solution of *hydrochloric acid*, heating to 40°, if necessary, and dilute to 20.0 ml with the same acid. The solution is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 12.5 mg of the substance under examination in 25.0 ml of mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 100.0 ml with mobile phase A.

Reference solution (b). Dissolve 2.5 mg of *famotidine impurity D IPRS [3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl] propanamide IPRS]* in *methanol* and dilute to 10.0 ml with the same solvent. To 1.0 ml of the solution, add 0.5 ml of the test solution and dilute to 100.0 ml with mobile phase A.

Reference solution (c). Dissolve 5 mg of *famotidine for system suitability IPRS* (famotidine impurities A, B, C, D, E, F, G) in mobile phase A and dilute to 10.0 ml with mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 50°,
- mobile phase: A. a mixture of 6 volumes of *methanol*, 94 volumes of *acetonitrile* and 900 volumes of a 0.1882 per cent w/v solution of *sodium hexanesulphonate* previously adjusted to pH 3.5 with *acetic acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Flow rate (ml per min.)
0	100	0	1
23	96	4	1
27	96	4	2
47	78	22	2
50	100	0	2
54	100	0	1

Inject reference solution (c). The relative retention time with reference to famotidine for 3-[[[2-[(diaminomethylene)amino] thiazol-4-yl]methyl]sulphanyl]propanamide (famotidine impurity D) is about 1.1, for 3-[[[2-[(diaminomethylene) amino] amino]thiazol-4-yl]methyl]sulphanyl]-N-sulphamoyl-propanamide (famotidine impurity C) is about 1.2, for N-

cyano-3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]propanimidamide (famotidine impurity G) is about 1.4, for 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]propanoic acid (famotidine Impurity F) is about 1.5, for 3-[[[2-[(diaminomethylene)amino]thiazol-4-yl]methyl]sulphanyl]propanimidamide (famotidine impurity A) is about 1. 6, for 3,5-bis[2-[[[2-[(diaminomethylene) amino] thiazol-4-yl]methyl]sulphanyl]ethyl]-4H-1,2,4,6-thiatriazine 1,1-dioxide (famotidine impurity B) is about 2.0, for 2,2'-[disulphanediyl bis (methylenethiazole-4,2-diyl)] diguanidine (famotidine Impurity E) is about 2.1.

For calculation of contents, multiply the areas of each known impurity by its correction factor, for impurity A=1.9, impurity B=2.5, impurity C=1.9, impurity F=1.7, impurity G=1.4 .

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to famotidine and famotidine impurity D is not less than 3.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of each secondary peak due to famotidine impurity A and G is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and for each famotidine impurity B,C,D and E is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), and the area of the peak due to famotidine impurity F is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); the sum of the areas of all other secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent); Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 80° at a pressure not exceeding 0.67 kPa for 5 hours.

Assay. Weigh 0.12 g, dissolve in 60 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01687 g of $C_8H_{15}N_7O_2S_3$.

Storage. Store protected from light.

Famotidine Tablets

Famotidine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of famotidine, $C_8H_{15}N_7O_2S_3$.

Usual strengths. 20 mg; 40 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2 volumes of *13.5 M ammonia*, 20 volumes of *toluene*, 25 volumes of *methanol* and 40 volumes of *ethyl acetate*.

Test solution. Shake a quantity of the powdered tablets containing about 40 mg of Famotidine with 4 ml of *glacial acetic acid*, dilute to 10 ml with the same solvent, centrifuge and use the clear supernatant liquid.

Reference solution. A 0.4 per cent w/v solution of *famotidine IPRS* in *glacial acetic acid*.

Apply to the plate 10 µl of each solution. Dry the plate in air and examine at 254 nm, The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of phosphate buffer prepared by dissolving 13.61 g of *potassium dihydrogen orthophosphate* in *water*, adjusted to pH 4.5 with *orthophosphoric acid* or *1 M potassium hydroxide*, add sufficient *water* to produce 1000 ml,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate suitably to obtain a solution containing 0.001 per cent w/v of Famotidine with the dissolution medium.

Reference solution. A 0.001 per cent w/v solution of *famotidine IPRS* in the dissolution medium.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{15}N_7O_2S_3$ in the tablet.

Q. Not less than 70 per cent of the stated amount of $C_8H_{15}N_7O_2S_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 0.05 M potassium dihydrogen phosphate, adjusted to pH 6.0 with *1 M potassium hydroxide.*

Test solution. Shake a quantity of intact tablets containing about 0.2 g of Famotidine, with 200 ml of the solvent mixture, add 200 ml of *methanol*. Shake for 60 minutes and dilute to 500 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 10.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 50.0 ml with the same solvent.

Reference solution (c). Dissolve 2 mg each of *3-[[[2-[(diaminomethylene)amino]-1,3-thiazol-4- yl]methyl] sulphinyl]-N-sulphamoylpropanamidine IPRS (famotidine impurity C IPRS)*, *3-[2-(Diaminomethylene-amino)-1,3-thiazol-4-ylmethylthio]propanoic acid IPRS (famotidine degradation impurity 1)* and *3-[2-(Diaminomethyleneamino)-1,3-thiazol-4-ylmethylthio] propanamide IPRS (famotidine degradation impurity 2)* in 40 ml of *acetonitrile*. Add 40 ml of *methanol* and dilute to 200.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 5.0 ml with the solvent mixture.

Reference solution (d). Dissolve 8 mg of *famotidine IPRS* in 20 ml of solvent mixture (solution A). To 1.0 ml of the solution, add 0.05 ml of *hydrogen peroxide solution* (generates famotidine degradation impurity 3).

Reference solution (e). Dilute 1.0 ml of solution A with 100.0 ml of the solvent mixture. Further dilute a suitable volume of the solution with an equal volume of reference solution (c).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-2),
- column temperature: 40°,
- mobile phase: a mixture of 7 volumes of *acetonitrile* and a mixture of 93 volumes of *0.1 M sodium acetate* containing 0.1 per cent v/v of *triethylamine*, adjusted to pH 6.0 with *glacial acetic acid*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 50 µl.

Inject reference solution (e). The test is not valid unless the resolution between the peaks due to famotidine and famotidine impurity C is not less than 1.4 and between the peaks due to famotidine and famotidine degradation impurity 2 is not less than 1.4.

Inject reference solution (a), (b), (c) and (d) and the test solution. In the chromatogram obtained with the test solution the areas of peaks corresponding to famotidine impurity C or famotidine degradation impurities 1 and 2 is not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (c) (0.5 per cent each), the area of peak corresponding to famotidine degradation impurity 3 is not more than the area of the peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 12.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of intact tablets containing about 0.2 g of Famotidine with 200 ml of the solvent mixture, add 200 ml of *methanol*. Shake for 60 minutes and dilute to 500 ml with the solvent mixture. Dilute 1.0 ml of the solution to 5.0 ml with the solvent mixture.

Reference solution. Dissolve 8 mg of *famotidine IPRS* in 4 ml of *methanol*, dilute to 20 ml with the solvent mixture. Dilute 1.0 ml of the solution to 5.0 ml with the solvent mixture.

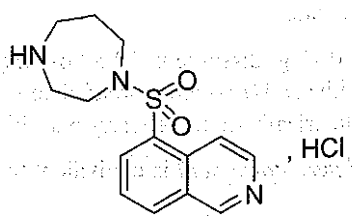
Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of famotidine, $C_8H_{15}N_7O_2S_3$.

Fasudil Hydrochloride



$C_{14}H_{17}N_3O_2S,HCl$ Mol Wt. 327.8

Fasudil Hydrochloride is 5-(1,4-Diazepane-1-sulfonyl) isoquinoline hydrochloride.

Fasudil Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{17}N_3O_2S,HCl$, calculated on the anhydrous basis.

Category. Cerebral Vasodilator.

Description. An off-white to creamy-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fasudil hydrochloride IPRS* or with the reference spectrum of fasudil hydrochloride.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with mobile phase.

Reference solution (a). A 0.0075 per cent w/v solution of *isoquinoline-5-sulphonic acid IPRS* in the mobile phase.

Reference solution (b). Dissolve 50 mg of *fasudil hydrochloride IPRS* in the mobile phase,add 1.0 ml of reference solution (a) and dilute to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of *water*, 15 volumes of *acetonitrile* and 0.5 volume of *trifluoroacetic acid*, adjusted to pH 7.0 with *triethylamine*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

The relative retention time for isoquinoline-5-sulphonic acid with respect to fasudil is about 0.25

Inject reference solution (b). The test is not valid unless the resolution between fasudil and isoquinoline-5-sulphonic acid is not less than 10.0, the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of peak due to isoquinoline-5-sulphonic acid is not more than 0.5 per cent, the area of any other secondary peak is not more than 0.5 per cent and and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

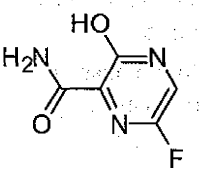
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 2.5 to 3.5 per cent, determined on 0.5 g.

Assay. Dissolve 0.3 g and 0.6 g *of mercuric acetate* in 40.0 ml of *glacial acetic acid*, add 15 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03278 g of $C_{14}H_{17}N_3O_2S,HCl$.

Favipiravir



$C_5H_4FN_3O_2$ Mol. Wt. 157.1

Favipiravir is 6-fluoro-3-hydroxypyrazine-2-carboxamide.

Favipiravir contains not less than 98.0 per cent and not more than 102.0 per cent of $C_5H_4FN_3O_2$, calculated on the anhydrous basis.

Category. Antiviral.

Description. An off-white to light yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *favipiravir IPRS* or with the reference spectrum of favipiravir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of *water* and 10 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture, with the aid of ultrasound and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.00015 per cent w/v of *6-chloro-3-hydroxy amide IPRS* and 0.1 per cent w/v of *favipiravir IPRS* in the solvent mixture.

Reference solution (b). A 0.0001 per cent w/v solution of *favipiravir IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (Such as Inert Sustain),
- column temperature: 35°,
- sample temperature: 5°,
- mobile phase: A. a 0.27 per cent w/v solution of *potassium dihydrogen orthophosphate* in *water*, adjusted to pH 2.5 with *orthophosphoric acid*,
  B. a mixture of 70 volumes of *acetonitrile* and 30 volumes of mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 300 nm and 365 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	89	11
6	89	11
14	72	28
24	26	74
35	26	74
38	3	97
47	3	97
48	89	11
55	89	11

Name	Relative retention time	Correction factor
Favipiravir acid impurity	0.52	1.04
Favipiravir (Retention time about 15 minutes)	1.0	—
6-chloro-3-hydroxy-amide impurity	1.17	0.70
6-bromo-3-hydroxy-amide impurity	1.29	0.81
6-fluoro-3-hydroxy-nitrile impurity	1.53	1.64

Inject reference solution (a) and (b) at 300 nm. The test is not valid unless resolution between the peaks due to favipiravir and 6-chloro-3-hydroxy-amide is not less than 2.0 in the chromatogram obtained with reference solution (a), the column efficiency is not less than 15000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution at 300 nm. In the chromatogram obtained with the test solution, the area of any peak corresponding to favipiravir acid, 6-fluoro-3-hydroxy-nitrile and any other secondary peak, each of, is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak corresponding to 6-chloro-3-hydroxy amide and 6-bromo-3-hydroxy amide.

Inject reference solution (b) and the test solution at 365 nm. In the chromatogram obtained with the test solution, the area of any peak corresponding to 6-chloro-3-hydroxy amide and 6-bromo-3-hydroxy amide, each of, is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The sum of all secondary peaks at 300 nm and 365 nm is not more than 1.0 per cent.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals. Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of *water* and 10 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *favipiravir IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3V),
- sample temperature: 8°,
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 30 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 321 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3500 theoretical plate, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of the $C_5H_4FN_3O_2$.

Storage. Store protected from moisture, at a temperature between 2° to 8°.

Favipiravir Tablets

Favipiravir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of favipiravir $C_5H_4FN_3O_2$.

Usual strengths. 200 mg; 400 mg.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *favipiravir IPRS* or with the reference spectrum of favipiravir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a buffer solution prepared by dissolving 2.99 g of *sodium acetate trihydrate* in 500 ml of *water*, add 14 ml of *2M glacial acetic acid* and dilute to 1000 ml with *water*, adjusted to pH 4.5 with *2M glacial acetic acid* or *2M sodium hydroxide*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.055 per cent w/v solution of *favipiravir IPRS* in *acetonitrile*. Dilute 10.0 ml of the solution to 25.0 ml with the dissolution medium.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 8°,
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 30 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 321 nm,
- injection volume: 5 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_5H_4FN_3O_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_5H_4FN_3O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of *water* and 10 volumes of *acetonitrile*.

Test solution. Disperse a quantity of powdered tablets containing 50 mg of Favipiravir in 40 ml of the solvent mixture, with the aid of ultrasound for 30 minutes with intermittent shaking and dilute to 50.0 ml with the solvent mixture and filter.

Reference solution. A 0.0005 per cent w/v solution of *fevipiravir IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inert Sustain),
- column temperature: 40°,
- sample temperature: 8°,
- mobile phase: A. a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* into 1000 ml of *water*, adjusted to pH 2.4 with *orthophosphoric acid*,

  B. 70 volumes of *acetonitrile* and 30 volumes of mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	89	11
6	89	11
14	72	28
24	26	74
35	26	74
38	3	97
47	3	97
48	89	11
55	89	11

Name	Relative retention time	Correction factor
Favipiravir acid impurity or acid impurity	0.53	1.08
Favipiravir	1.0	—
6-chloro-3-hydroxy-amide impurity or chlorohomolog impurity*	1.27	—
6-bromo-3-hydroxy-amide impurity*	1.43	—
6-fluoro-3-hydroxy-nitrile impurity*	1.73	—

*Process impurity included for identification only and not included in the calculation of total degradation products.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 10000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to favipiravir acid impurity is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of *water* and 10 volumes of *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.4 g of Favipiravir in 10 ml of the *water*, with the aid of ultrasound with intermittent shaking, add 10 ml of *acetonitrile* and 50 ml of equal volumes of *acetonitrile* and *water*, sonicate for 15 minutes and dilute to 100.0 ml with equal volumes of *acetonitrile* and *water*, filter. Dilute 5.0 ml of the solution to 200.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *favipiravir IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Intersil ODS-3V),
- sample temperature: 8°,
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 30 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 321 nm,
- injection volume: 5 µl.

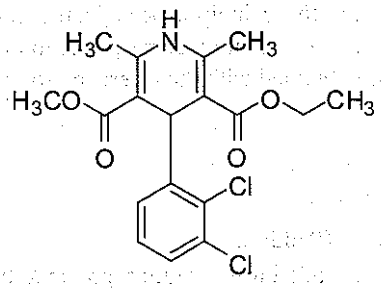
Inject the reference solution. The test is not valid unless the column efficiency is not less than 3500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of the $C_5H_4FN_3O_2$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Felodipine



$C_{18}H_{19}Cl_2NO_4$ Mol. Wt. 384.3

Felodipine is ethyl methyl (4*RS*)-4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

Felodipine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{19}Cl_2NO_4$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or light yellow crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *felodipine IPRS* or with the reference spectrum of felodipine.

B. When examined in the range 220 nm to 400 nm (2.4.7), a 0.0015 per cent w/v solution in *methanol*, shows absorption maxima at about 238 nm and 361 nm; the absorbance ratio of 361 nm and 238 nm is between 0.34 to 0.36.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *ethyl acetate* and 60 volumes of *cyclohexane*.

Test solution. Dissolve 10 mg of the substance under examination in 10.0 ml of *methanol*.

Reference solution (a). Dissolve 10 mg of *felodipine IPRS* in 10.0 ml of *methanol*.

Reference solution (b). Dissolve 5 mg of *nifedipine IPRS* in 5.0 ml of reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under

ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

D. Dissolve 0.15 g in a mixture of 25 ml of *2-methyl-2-propanol* and 25 ml of *perchloric acid solution*. Add 10 ml of *0.1 M cerium sulphate*, allow to stand for 15 minutes, add 3.5 ml of *strong sodium hydroxide solution* and neutralise with *dilute sodium hydroxide solution*. Shake with 25 ml of *dichloromethane*, evaporate the lower layer to dryness on a water-bath under nitrogen (the residue is also used in the test for related substances). Dissolve 20 mg of the residue in 50.0 ml of *methanol*, Dilute 2 .0 ml of the solution to 50.0 ml with *methanol*. When examined in the range 220 nm to 400 nm (2.4.7), the resulting solution shows an absorption maximum only at about 273 nm.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *methanol* (Solution A) is clear (2.4.1).

Light absorption. Not more than 0.1, determined at 440 nm (2.4.7) on solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c). Dissolve 10 mg of the residue obtained in identification test D [ethyl methyl 4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate] (felodipine impurity A) and 5 mg of *felodipine IPRS* in the mobile phase, then dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *methanol*, 40 volumes of *acetonitrile* and 40 volumes of a *phosphate buffer solution pH 3.0* containing 0.08 per cent w/v of *orthophosphoric acid* and 0.8 per cent w/v of *sodium dihydrogen phosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to felodipine and felodipine impurity A is not less than 2.5. The order of elution is dimethyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, (felodipine impurity B), ethyl methyl 4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate (felodipine impurity A), felodipine and diethyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (felodipine impurity C).

Inject reference solution (a), (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of secondary peaks corresponding to felodipine impurity B and C is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). Sum of all other secondary peaks is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.16 g, dissolve in a mixture of 25 ml of *2-methyl-2-propanol* and 25 ml of *1 M perchloric acid*. Titrate slowly with *0.1 M cerium ammonium sulphate* until the pink colour disappears using 0.05 ml of *ferroin sulphate solution* as an indicator.

1 ml of *0.1 M cerium ammonium sulphate* is equivalent to 0.01921 g of $C_{18}H_{19}Cl_2NO_4$.

Storage: Store protected from light.

Felodipine Prolonged-release Tablets

Felodipine Sustained-release Tablets; Felodipine Extended-release Tablets

Felodipine Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Felodipine Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of felodipine, $C_{18}H_{19}Cl_2NO_4$.

Usual strengths. 2.5 mg; 5 mg; 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Proceed as directed in the Assay, except that after centrifuging a portion of the solution at high speed for 15 minutes, filter.

Reference solution (a). A 0.02 per cent w/v solution of *felodipine impurity A IPRS* in *methanol.* Dilute 10.0 ml of the solution to 100 ml with the mobile phase.

Reference solution (b). A 0.2 per cent w/v solution of *felodipine IPRS* in *methanol.*

Reference solution (c). Dilute 15.0 ml of reference solution (a) and 5.0 ml of reference solution (b) to 100 ml with the mobile phase.

Reference solution (d). Dilute 10.0 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture 40 volumes of a buffer solution prepared by dissolving 6.9 g of *monobasic sodium phosphate* in about 800 ml of *water*, adjusted to pH 3.0 with *orthophosphoric* acid and dilute to 1000 ml with *water*, 40 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 40 µl.

Inject reference solution (c). The test is not valid unless the relative retention time with reference to felodipine for felodipine impurity A is about 0.75 and the resolution between the peaks due to felodipine and felodipine impurity A is not less than 1.5 and the theoretical plates is not less than 1500.

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to felodipine impurity A is not more than 2.0 per cent the area of the principal peak in the chromatogram obtained with reference solution (d).

Uniformity of content. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Sonicate 1 tablet with 40 ml of *acetonitrile* for 20 minutes and add 20 ml of *methanol*, and then shake by mechanical means for 30 minutes. Allow to cool to room temperature, dilute to 100.0 ml with the buffer solution. Centrifuge for 15 minutes. Dilute a portion of the supernatant with mobile phase to obtain a solution containing about 20 µg of felodipine per ml, filter.

Inject 40 µl of the reference solution and the test solution.

Calculate the content of felodipine, $C_{18}H_{19}Cl_2NO_4$ in the Tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 6.9 g of *monobasic sodium phosphate* in about 800 ml of *water,* adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water.*

Test solution. Weigh and powder 10 tablets. Disperse a quantity of powder containing about 10 mg of Felodipine with 40 ml of *acetonitrile* and 20 ml of *methanol,* and sonicate for 5 minutes. Add about 30 ml of buffer solution, and shake by mechanical means for 30 minutes. Allow the solution to cool to room temperature, dilute to 100.0 ml with buffer solution. Centrifuge for 15 minutes. Dilute 10 ml of the supernatant to 50 ml with the mobile phase, filter.

Reference solution (a). A 0.2 per cent w/v solution of *felodipine IPRS* in *methanol.*

Reference solution (b). Dilute a volume of reference solution (a) with mobile phase to obtain a solution having a concentration of 0.02 mg per ml.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of buffer solution, 40 volumes of *acetonitrile* and 20 volumes of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 362 nm,
- injection volume: 40 µl.

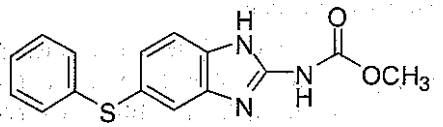
Inject reference solution (b). The test is not valid unless the column efficiency is not less than 1500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of felodipine, $C_{18}H_{19}Cl_2NO_4$ in the tablets.

Storage. Store protected from moisture.

Fenbendazole



$C_{15}H_{13}N_3O_2S$ Mol. Wt. 299.4

Fenbendazole is methyl 5-(phenylthio)-2-benzimidazolecarbamate.

Fenbendazole contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{15}H_{13}N_3O_2S$, calculated on the dried basis.

Category. Anthelminthic.

Description. A white or almost white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fenbendazole IPRS* or with the reference spectrum of fenbendazole.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 10.0 ml of *methanolic hydrochloric acid.*

Reference solution (a). Dissolve 50 mg of *fenbendazole IPRS* in 10.0 ml of *methanolic hydrochloric acid.* Dilute 1.0 ml of the solution to 200.0 ml with *methanol.* Dilute 5.0 ml of the solution to 10.0 ml with *methanolic hydrochloric acid.*

Reference solution (b). Dissolve 10 mg of *methyl (1H-benzimidazol-2-yl)carbamate IPRS (fenbendazole impurity A IPRS)* in 100.0 ml of *methanol.* Dilute 1.0 ml of the solution to 10.0 ml with *methanolic hydrochloric acid.*

Reference solution (c). Dissolve 10 mg of *methyl (5-chloro-1H-benzimidazol-2-yl)carbamate IPRS (fenbendazole impurity B IPRS)* in 100.0 ml of *methanol.* Dilute 1.0 ml of the solution to 10.0 ml with *methanolic hydrochloric acid.*

Reference solution (d). Dissolve 10 mg of *fenbendazole IPRS* and 10 mg of *mebendazole IPRS* in 100.0 ml of *methanol.* Dilute 1.0 ml of the solution to 10.0 ml with *methanolic hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 1 volume of *anhydrous acetic acid,* 30 volumes of *methanol* and 70 volumes of *water,*

B. a mixture of 1 volume of *anhydrous acetic acid,* 30 volumes of *water* and 70 volumes *methanol,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
10	0	100
40	0	100
50	100	0
55	100	0

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to fenbendazole and mebendazole is not less than 1.5.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak due to fenbendazole impurity A is not more than 2.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The area of secondary peak corresponding to fenbendazole impurity B is not more than 2.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of any other secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent); The sum of the areas of all other secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.3 per cent.

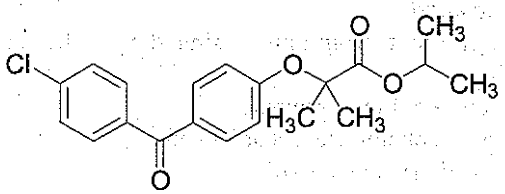
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.2 g in 30 ml of *anhydrous acetic acid,* warming gently, if necessary. Cool and titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02994 g of $C_{15}H_{13}N_3O_2S$.

Storage. Store protected from light.

Fenofibrate



$C_{20}H_{21}ClO_4$ Mol. Wt. 360.8

Fenofibrate is isopropyl [4-(4-chlorophenyl)-2-phenoxy-2-methyl]propionate

Fenofibrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{21}ClO_4$, calculated on the dried basis.

Category. Antihyperlipidemic.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fenofibrate IPRS* or with the reference spectrum of fenofibrate.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *acetone* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity. Dissolve 1.0 g in 50 ml of *ethanol (95 per cent)*, previously neutralized using 0.2 ml of *phenolphthalein* and titrate with *0.1 M sodium hydroxide*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the color of the indicator to pink.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *fenofibrate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v each of *fenofibrate IPRS*, *fenofibrate impurity A IPRS*, *fenofibrate impurity B IPRS* and 0.01 per cent w/v of *fenofibrate impurity G IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*, adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 286 nm,
- injection volume: 20 µl.

The relative retention time with reference to fenofibrate for (4-chlorophenyl)(4-hydroxyphenyl)methanone (fenofibrate impurity A) is about 0.34, for fenofibric acid (fenofibrate impurity B) is about 0.36, for (3*RS*)-3-[4-(4-chlorobenzoyl) phenoxy]butan-2-one (fenofibrate impurity C) is about 0.50, for methyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate (fenofibrate impurity D) is about 0.65, for ethyl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoate (fenofibrate impurity E) is about 0.80, for (4-chlorophenyl) [4-(1-methylethoxy)phenyl] methanone (fenofibrate impurity F) is about 0.85 and for 1-methylethyl 2-[[2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoyl]oxy]-2-methylpropanoate (fenofibrate impurity G) is about 1.35.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to fenofibrate impurity A and fenofibrate impurity B is not less than 1.5.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of each peak due to fenofibrate impurity A and B is not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of the peak due to fenofibrate impurity G is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides (2.3.12). 25 ml of solution A, complies with the limit test for chlorides (100 ppm).

Sulphates (2.3.17). Dissolve 5 g in 25 ml of *distilled water* and heat at 50° for 10 minutes. Cool and dilute to 50.0 ml with the same solvent, filter (Solution A). 15 ml of the solution complies with the limit test for sulphates (100 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent determined on 1 g by drying in vacuum over phosphorus pentoxide at 60°.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances using injection volume: 5 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{20}H_{21}ClO_4$.

Storage. Store protected from light.

Fenofibrate Capsules

Fenofibrate Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fenofibrate, $C_{20}H_{21}ClO_4$.

Usual strengths. 50 mg; 150 mg.

Identification

A. Transfer the contents of 1 Capsule to a glass centrifuge tube, add an amount of *dichloromethane*, equivalent to about 10 ml per 67 mg of fenofibrate and shake vigorously. Pass through a suitable paper filter into a separatory funnel, wash with *water* and collect the dichloromethane layer. Evaporate under a stream of nitrogen and dry under vacuum at 60° for 1 hour. Determine by infrared absorption spectrophotometry (2.4.6) on the residue. Compare the spectrum with that obtained with *fenofibrate IPRS* or with the reference spectrum of fenofibrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 1000 ml of *0.05 M sodium lauryl sulphate*,

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed of *fenofibrate IPRS* in the mobile phase and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of test solution.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}ClO_4$.

Q. Not less than 70 per cent of the stated amount of $C_{20}H_{21}ClO_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh the contents of 20 Capsules. Mix and transfer a weighed portion of the powder, containing about 67 mg of Fenofibrate sonicate with 80 ml of mobile phase for 10 minutes, stir for 15 minutes and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.067 per cent w/v of *fenofibrate IPRS* and 0.000335 per cent w/v of *fenofibrate impurity B IPRS* (*fenofibric acid IPRS*) in the mobile phase.

Reference solution (b). A solution containing 0.000335 per cent w/v each of *fenofibrate IPRS* and *fenofibrate impurity B IPRS* (*fenofibric acid IPRS*) in the mobile phase.

Use chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to fenofibrate and fenofibrate impurity B is not less than 3.0, the column efficiency for the peak due to fenofibrate impurity B is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to fenofibrate impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and sum of the areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh the contents of 20 Capsules. Mix and transfer the powder containing 67 mg of fenofibrate and dissolve in 80 ml of the mobile phase and dilute to 100 ml with the mobile phase. Dilute 5.0 ml of the solution to 50 ml with the mobile phase.

Reference solution. A 0.0067 per cent w/v solution of *fenofibrate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 80 volumes of *methanol* and 20 volumes of buffer solution prepared by dissolving 136 mg of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 2.9 with *dilute orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 6000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{21}ClO_4$ in the Capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Fenofibrate Tablets

Fenofibrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fenofibrate, $C_{20}H_{21}ClO_4$.

Category. Antihyperlipidmic.

Usual strengths. 48 mg; 54 mg; 120 mg; 145 mg; 160 mg; 200 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium: 1000 ml of *0.05 M sodium lauryl sulphate* in *water*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter of 0.45 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the medium. Measure the absorbance of the resulting solution at the maximum at about 292 nm (2.4.7). Calculate the content of fenofibrate, $C_{20}H_{21}ClO_4$ in the medium from the absorbance of a 0.0012 per cent w/v solution of *fenofibrate IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{21}ClO_4$ in the tablets.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse a quantity of powder tablets containing 50 mg of fenofibrate in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 5 mg each of *fenofibrate impurity A IPRS* and *fenofibrate impurity B IPRS* in *acetonitrile* and dilute to 100.0 ml with *acetonitrile*. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). A 0.00005 per cent w/v solution of *fenofibrate IPRS* in the mobile phase.

Inject reference solution (a). The test is not valid unless the resolution between the peaks corresponding to fenofibrate impurity A and fenofibrate impurity B is not less than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to fenofibrate impurity A and fenofibrate impurity B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than twice the area of the principal peak obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent).

Other tests. Complies with the tests stated under tablets.

Assay. Determine by liquid chromatography (2.4.14).

Acidified water. Adjust the pH of *water* with *orthophosphoric acid* to 2.5.

Mobile phase. A mixture of 70 volumes of *acetonitrile* and 30 volumes of acidified water.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of fenofibrate in 30 ml of acidified water with the aid of ultrasound, add 60 ml of *acetonitrile* and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). Dissolve 5 mg each of *fenofibrate impurity A IPRS* and *fenofibrate impurity B IPRS* in *acetonitrile* and dilute to 100.0 ml with *acetonitrile*. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). A 0.005 per cent w/v solution of *fenofibrate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 70 volumes of *acetonitrile* and 30 volumes of acidified water,

- flow rate: 1.2 ml per minute,
- spectrophotometer set at 286 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Fenofibrate impurity A[1]	0.34	0.7
Fenofibrate impurity B[2]	0.36	1.0
(3 RS)-3-[4-(4-Chlorobenzoyl) phenoxy]butan-2one*	0.50	---
Methyl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoate*	0.65	---
Ethyl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoate*	0.80	---
(4-Chlorophenyl)[4-(1-methylethoxy) phenyl]methanone*	0.85	---
Fenofibrate	1.00	---
Fenofibrate impurity C[3]	1.35	---

*It is a process impurity.

[1](4- chlorophenyl) (4-hydrxyphenyl) methanone,

[2]2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propanoic acid,

[3]1-methylethyl 2-[[2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoyl]-2-methylpropanoate,

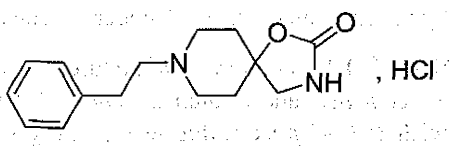
Inject reference solution (a) and reference solution (b). The test is not valid unless the resolution between the peaks corresponding to fenofibrate impurity A and fenofibrate impurity B is not less than 2.0 in the chromatogram obtained with reference solution (a) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_{20}H_{21}ClO_4$, in the tablets.

Storage. Store protected from moisture.

Fenspiride Hydrochloride



$C_{15}H_{20}N_2O_2$,HCl Mol Wt .296.8

Fenspiride Hydrochloride is 8-(2-Phenylethyl)-1-oxa-3,8-diazaspiro[4.5]decan-2-one hydrochloride.

Fenspiride Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{20}N_2O_2$,HCl, calculated on the dried basis.

Category. Bronchodilator.

Description. A white to almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fenspiride hydrochloride IPRS* or with the reference spectrum of fenspiride hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5 , determined on 1.0 per cent w/v solution of substance under examination in *water* .

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *fenspiride hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of buffer solution prepared to dissolving 3.0 g of *sodium dihydrogen orthophosphate* in 1000 ml of *water,* adjusted to pH 3.0 with *orthophosphoric acid* and 20 volumes of *methanol,*
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

The relative retention time for fensipiride impurity A is about 0.7 and for fensipiride impurity B is about 1.5 with respect to fensipiride hydrochloride.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any peak due to fenspiride impurity A(1-phenyl ethyl-4-hydroxyl-4-amino methyl piperide-2- hydrochloride) is not more than 0.1 per cent , the area of the any peak due to fenspiride impurity B (2-phenyl ethyl -1-oxo-3,8-diazospiro-4,5 -ecan-2one-n-oxide) is not more than 0.2 per cent , the area of any other secondary peak is not more than 0.1 per cent and the sum of areas of all the secondary peaks is not more than 0.5 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method A (10 ppm).

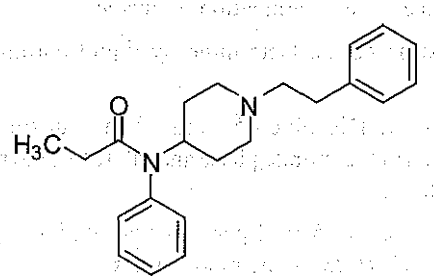
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.3 g by drying in an oven at 100° for 3 hours at a pressure of 0.67 kPa.

Assay. Dissolve 0.235 g in 60.0 ml of the 1.0 per cent *mercuric acetate solution* in *glacial acetic acid*. Titrate with *0.1 M perchloric acid* to determine the end-point potentiometrically (2.4.25).

1 ml of *0.1 M perchloric acid* is equivalent to 0.029679 g of $C_{15}H_{20}N_2O_2,HCl$.

Fentanyl



$C_{22}H_{28}N_2O$ Mol. Wt. 336.5

Fentanyl is *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]propanamide.

Fentanyl contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{22}H_{28}N_2O$, calculated on the dried basis.

Category. Analgesic.

Description. A white or almost white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fentanyl IPRS* or with the reference spectrum of fentanyl.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *methanol*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 20.0 ml with *methanol*.

Reference solution (b). Dissolve 10 mg of the substance under examination in 10.0 ml of *dilute hydrochloric acid*. Heat on a water-bath under a reflux condenser for 4 hours. Neutralise with 10.0 ml of *dilute sodium hydroxide solution* and evaporate to dryness on a water-bath, cool and dissolve the residue in 10.0 ml of *methanol*, filter.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a 0.5 per cent w/v solution of *ammonium carbonate* in a mixture of 10 volumes of *tetrahydrofuran* and 90 volumes of *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
15	40	60
20	40	60
25	90	10
30	90	10

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to fentanyl and fentanyl impurity D is not less than 8.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of secondary peaks corresponding to *N*-phenyl-*N*-[*cis,trans*-1-oxido-1-(2-phenylethyl)piperidin-4-yl]propanamide (fentanyl impurity A), *N*-phenyl-*N*-(piperidin-4-yl)propanamide (fentanyl impurity B), *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]acetamide (fentanyl impurity C), *N*-phenyl-1-(2-phenylethyl)piperidin-4-amine (fentanyl impurity D) is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

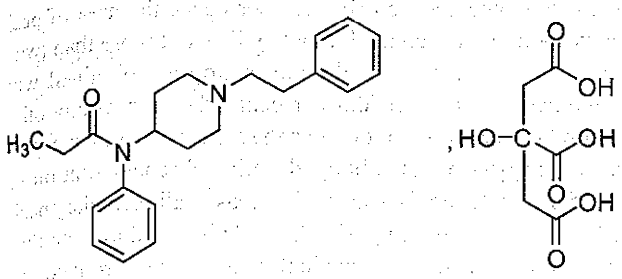
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 50° under vacuum.

Assay. Dissolve 0.2 g in 50 ml of a mixture of 1 volume of *anhydrous acetic acid* and 7 volumes of *methyl ethyl ketone*. Titrate with *0.1 M perchloric acid*, using 0.2 ml of *naphtholbenzein solution* as indicator, until the colour changes from orange-yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03365 g of $C_{22}H_{28}N_2O$.

Storage. Store protected from light.

Fentanyl Citrate



$C_{22}H_{28}N_2O,C_6H_8O_7$ Mol. Wt. 528.6

Fentanyl Citrate is *N*-(phenylethyl-4-piperidinyl)-*N*-phenylpropionamide citrate.

Fentanyl Citrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{22}H_{28}N_2O,C_6H_8O_7$, calculated on the dried basis.

Category. Analgesic.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fentanyl citrate IPRS* or with the reference spectrum of fentanyl citrate.

B. Melting point (2.4.21). 152°.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *methanol.*

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol.* Dilute 5.0 ml of the solution to 20.0 ml with *methanol.*

Reference solution (b). Dissolve 10 mg of the substance under examination in 10.0 ml of *dilute hydrochloric acid.* Heat on a water-bath under a reflux condenser for 4 hours. Neutralise with 10.0 ml of *dilute sodium hydroxide solution* and evaporate to dryness on a water-bath, cool and dissolve the residue in 10.0 ml of *methanol* and filter. To prepare degradation compound *N*-phenyl-1-(2-phenylethyl)piperidin-4-amine (fentanyl citrate impurity D).

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. 0.5 per cent w/v solution of *ammonium carbonate* in a mixture of 10 volumes of *tetrahydrofuran* and 90 volumes of *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
15	40	60
20	40	60
25	90	10
30	90	10

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to fentanyl and fentanyl impurity D is not less than 3.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 50° under vacuum.

Assay. Weigh 0.3 g, dissolve in 50 ml of a mixture of 1 volume of *anhydrous acetic acid* and 7 volumes of *methyl ethyl ketone.* Titrate with *0.1 M perchloric acid,* using 0.2 ml of *naphtholbenzein solution* as indicator, until the colour changes from orange-yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05286 g of $C_{22}H_{28}N_2O,C_6H_8O_7$.

Storage. Store protected from light.

Fentanyl Injection

Fentanyl Citrate Injection

Fentanyl Injection is a sterile solution of Fentanyl Citrate in Water for Injections.

Fentanyl Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fentanyl, $C_{22}H_{28}N_2O$.

Usual strength. 50 µg per ml.

Identification

A. When examined in the range 230 to 350 nm (2.4.7), a solution containing 0.005 per cent w/v of fentanyl exhibits two maxima at 251 and 257 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak obtained with the reference solution.

C. Gives reaction A for citrates (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing about 0.5 mg of Fentanyl to 10.0 ml with the mobile phase, if necessary.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b). A 0.00005 per cent w/v solution of *N-phenyl-N-[cis,trans-1-oxido-1-(2-phenylethyl)piperidin-4-yl]propanamide IPRS (fentanyl impurity A IPRS)* in the mobile phase.

Reference solution (c). Dissolve 10 mg of *fentanyl citrate IPRS* in 10 ml of *2 M hydrochloric acid*, heat on a water-bath under a reflux condenser for 4 hours and neutralise with 10 ml of *2 M sodium hydroxide*. Evaporate to dryness on a water-bath, cool, dissolve the residue in 10 ml of *methanol* and filter. Dilute 1 ml of the filtrate to 10 ml with the mobile phase {generation of *N*-phenyl-1-(2-phenylethyl)piperidin-4-amine (fentanyl impurity D)}.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with endcapped octadecylsilane bonded to porous silica (10 µm) (Such as Bondclone C18),
- mobile phase: a solution containing 0.3 per cent w/v of *potassium dihydrogen phosphate* in a mixture of 4 volumes of *acetonitrile*, 40 volumes of *methanol* and 56 volumes of *water*, adjusted to pH 3.2 with *ortho-phosphoric acid*,
- flow rate: 1.25 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 100 µl.

Inject reference solution (c).The relative retention time with reference to fentanyl for fentanyl impurity D is about 0.8.

Inject reference solution (a), (b) and the test solution. Run the chromatogram twice the retention time of the principal peak: In the chromatogram obtained with test solution the area of peak corresponding to fentanyl impurity A is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of peak corresponding to fentanyl impurity D is not more than twice the area of the peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent) and the sum of the areas of all secondary peaks other than fentanyl impurity A and D is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Bacterial endotoxins (2.2.3). Not more than 50.0 Endotoxin Units per mg.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing about 0.5 mg of Fentanyl to 10.0 ml with the mobile phase, if necessary.

Reference solution. A 0.008 per cent w/v solution of *fentanyl citrate IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

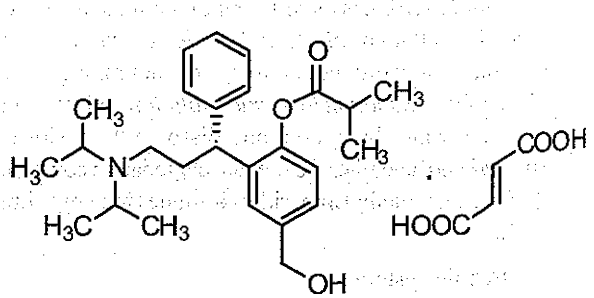
Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{28}N_2O$ in the injection.

Storage. Store protected from light.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of fentanyl.

Fesoterodine Fumarate



$C_{30}H_{41}NO_7$ Mol Wt. 527.7

Fesoterodine Fumarate is 2-((*R*)-3-(Diisopropylamino)-1-phenylpropyl)-4-(hydroxymethyl)phenyl isobutyrate fumarate.

Fesoterodine Fumarate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{30}H_{41}NO_7$, calculated on the anhydrous basis.

Category. Anticholinergic.

Description. A white to off white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fesoterodine fumarate IPRS* or with the reference spectrum of fesoterodine fumarate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +5.0° to +7.0°, determined in 1.0 per cent w/v solution in *ethanol* at 20°.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of mobile phase A and 40 volumes of *acetonitrile.*

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution. A 0.001 per cent w/v solution of *fesoterodine fumarate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 2.72 g of *potassium dihydrogen orthophosphate* into 1000 ml of *water*, adjusted to pH 6.5 with *potassium hydroxide*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
20	60	40
45	40	60
50	40	60
53	60	40
60	60	40

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of area of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of mobile phase A and 40 volumes of *acetonitrile.*

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 25.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.02 per cent w/v solution of *fesoterodine fumarate IPRS* in the solvent mixture.

Chromatographic system

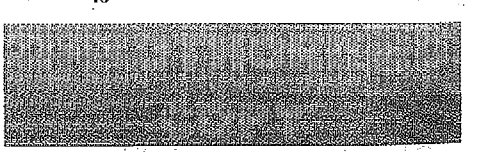
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 2.72 g of *potassium dihydrogen orthophosphate* into 1000 ml of *water*, adjusted to pH 6.5 with *potassium hydroxide*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	55	45
20	55	45
25	40	60
30	40	60
32	55	45
40	55	45

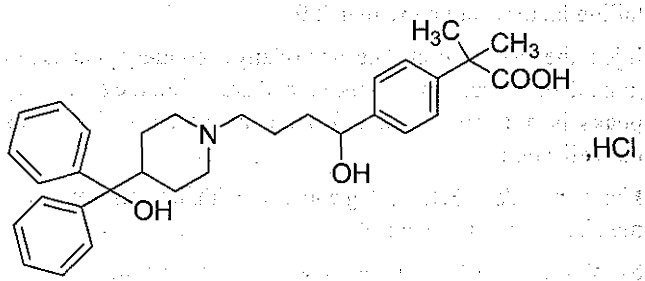
Inject the reference solution. The test is not valid unless the column efficiency is not less than 3500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{30}H_{41}NO_7$.



Fexofenadine Hydrochloride



$C_{32}H_{39}NO_4,HCl$ Mol. Wt. 538.1

Fexofenadine Hydrochloride is (*RS*) α,α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1 piperidinyl] butyl] benzene acetic acid hydrochloride.

Fexofenadine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{32}H_{39}NO_4,HCl$, calculated on the anhydrous basis.

Categrory. Antihistaminic.

Description. A white to off-white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fexofenadine hydrochloride IPRS* or with the reference spectrum of fexofenadine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Fexofenadine impurity B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A solution containing 0.025 per cent w/v of *fexofenadine hydrochloride IPRS* and 0.0005 per cent w/v of *{3-[1-hydroxy-4-[4-(hydroxydiphenyl-methyl)-1-piperidinyl]butyl]-α,α-dimethylbenzeneacetic acid hydrochloride} IPRS* (*fexofenadine impurity B IPRS*) in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with beta cyclodextrin bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *ammonium acetate buffer* prepared by mixing 1.15 ml of *glacial acetic acid* in 1000 ml of *water*, adjusted to pH 4.0 with *6 M ammonium hydroxide* and 20 volumes of *acetonirile*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (a). The relative retention time with respect to fexofenadine, for fexofenadine impurity B is about 0.7 and the resolution between fexofenadine and fexofenadine impurity B is not less than 3.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to fexofenadine impurity B is not more than the area of the peak due to fexofenadine impurity B in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Use the phosphate-perchlorate buffer, solvent mixture and chromatographic system as described under Assay.

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the solvent mixture.

Reference solution. A solution containing 0.0005 per cent w/v, of each, *fexofenadine hydrochloride IPRS* and *[benzeneacetic acid, 4-(1-oxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl] butyl]-α,α-dimethyl] IPRS* (*fexofenadine impurity A IPRS*) in the mobile phase.

Inject the reference solution. The test is not valid unless the resolution between fexofenadine and fexofenadine impurity A is not less than 10, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections for both peaks is not more than 2.0 per cent and 3.0 per cent respectively.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to the peak of fexofenadine impurity A is not more than 0.4 times the area of the corresponding peak in the chromatogram obtained with reference solution (0.2 per cent), area of any peak due to decarboxylated degradant [(+)-4-[1-hydroxy-4-(hydroxydi-phenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene], with a relative retention time of 3.2 with respect to fexofenadine hydrochloride, is not more than 0.3 times the area of the peak due to fexofenadine hydrochloride in the chromatogram obtained with reference solution (0.15 per cent) and the area of any secondary peak is not more than 0.2 times the area of the peak due to fexofenadine hydrochloride in the chromatogram obtained with reference solution (0.1 per cent). The sum of all the impurities is not more than 0.5 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides. 6.45 per cent to 6.75 per cent.

Dissolve about 0.3 g of the substance under examination in 50 ml of *methanol*. Titrate with *0.1 M silver nitrate*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.00354 g of chloride.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent for the anhydrous form and 6.0 per cent to 10.0 per cent for the hydrate form (dihydrate and trihydrate forms of fexofenadine hydrochloride), determined on 1 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *phosphate-perchlorate buffer*.

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the solvent mixture. Dilute 3.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A solution containing 0.006 per cent w/v of *fexofenadine hydrochloride IPRS* and 0.0005 per cent w/v of *fexofenadine impurity A IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *phosphate-perchlorate buffer* prepared by dissolving 6.64 g of *monobasic sodium phosphate* and 0.84 g of *sodium perchlorate* in 1000 ml of *water*, adjusted to pH 2.0 with *orthophosphoric acid* and 35 volumes of *acetonitrile*. Add 3 ml of *triethylamine* to 1000 ml of the mixture,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between fexofenadine and fexofenadine impurity A is not less than 10, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections for both peaks is not more than 2.0 per cent and 3.0 per cent respectively.

Inject the reference solution and the test solution.

Calculate the content of $C_{32}H_{39}NO_4$, HCl.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Fexofenadine Capsules

Fexofenadine Hydrochloride Capsules

Fexofenadine Capsules contain not less than 93.0 per cent and not more than 105.0 per cent of the stated amount of fexofenadine hydrochloride, $C_{32}H_{39}NO_4$,HCl.

Usual strengths. 120 mg; 180 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 15 and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). A 0.007 per cent w/v solution of *fexofenadine hydrochloride IPRS* in the dissolution medium.

NOTE — A small amount of methanol, not exceeding 0.5 per cent of the total volume, can be used to dissolve fexofenadine hydrochloride.

Reference solution (b). A solution containing 0.001 per cent w/v of *benzene acetic acid-4-[1-oxy-4(4-(hydroxydiphenyl-methyl)-1-piperidinyl]butyl)-α,α-dimethyl IPRS (fexofenadine impurity A IPRS)* and 0.006 per cent w/v of *fexofenadine hydrochloride IPRS* in *water*.

NOTE — A small amount of acetic acid, not exceeding 5 per cent of the total volume, can be used to dissolve fexofenadine impurity A.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 1.0 g of *monobasic sodium phosphate*, 0.5 g of *sodium perchlorate*, and 0.3 ml of *orthophosphoric acid* in 300 ml of *water* and 70 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to fexofenadine and fexofenadine impurity A is not less than 2.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Q. Not less than 50 per cent in 15 minutes and not less than 75 per cent in 45 minutes, of the stated amount of $C_{32}H_{39}NO_4$,HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Phosphate-perchlorate buffer. Dissolve 6.64 g of *monobasic sodium phosphate* and 0.84 g of *sodium perchlorate* in 1000 ml of *water*. Adjusted to pH 2.0 with *orthophosphoric acid*.

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *phosphate-perchlorate buffer*.

Test solution (a). Weigh a quantity of the mixed contents of 20 capsules containing about 50 mg of Fexofenadine Hydrochloride, add 40 ml of solvent mixture and shake by mechanical means for 60 minutes. Mix with the aid of ultrasound for about 2 minutes. Cool and dilute to 50.0 ml with the solvent mixture.

Test solution (b). Dilute 3.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution. A solution containing 0.006 per cent w/v of *fexofenadine hydrochloride IPRS* and 0.0005 per cent w/v of *fexofenadine impurity A IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *phosphate-perchlorate buffer* and 35 volumes of *acetonitrile*. Add 0.3 ml of *triethylamine* and mix,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution.The test is not valid unless the resolution between fexofenadine and fexofenadine impurity A is not less than 10, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections determined from fexofenadine and fexofenadine impurity A are not more than 2.0 per cent and 3.0 per cent respectively.

Inject the reference solution and test solution (a). In the chromatogram obtained with test solution (a) the area due to fexofenadine impurity A is not more than 0.8 times the area of the peak due to fexofenadine impurity A in the chromatogram obtained with the reference solution (0.4 per cent), the area of the peak due to decarboxilated degradant [(+)-4-[1-hydroxy-4-[4-(hydroxydi-phenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene having a relative retention time of 3.2 is not more than 0.03 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of any other individual impurity is not more than 0.03 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), and the sum of all the impurities is not more than 0.5 per cent.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject the reference solution and test solution (b).

Calculate the content of $C_{32}H_{39}NO_4$, HCl in the capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Fexofenadine Tablets

Fexofenadine Hydrochloride Tablets

Fexofenadine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fexofenadine hydrochloride, $C_{32}H_{39}NO_4$,HCl.

Usual strengths. 120 mg; 180 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.001 M hydrochloric acid*,
Speed and time. 50 rpm and 10 and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). Dissolve a weighed quantity of *fexofenadine hydrochloride IPRS* in the dissolution medium to obtain a solution having a known concentration similar to that expected for the solution under test.

NOTE — A small amount of methanol, not exceeding 0.5 per cent of the total volume, can be used to dissolve fexofenadine hydrochloride.

Reference solution (b). A 0.044 per cent w/v solution of *benzene acetic acid,-4-[1-oxy-4(4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl)-α,α-dimethyl IPRS (fexofenadine impurity A IPRS)* in *water*. To 1.0 ml of the solution add 40 ml of reference solution (a).

NOTE — A small amount of acetic acid, not exceeding 5 per cent of the total volume, can be used to dissolve fexofenadine impurity A.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 1.0 g of *monobasic sodium phosphate*, 0.5 g of *sodium perchlorate*, and 0.3 ml of *orthophosphoric acid* in 300 ml of *water* and 70 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (b). The resolution between fexofenadine and fexofenadine impurity A is not less than 2.0.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution (a) and the test solution (a).

Q. Not less than 60 per cent in 10 minutes and not less than 80 per cent in 30 minutes, of the stated amount of $C_{32}H_{39}NO_4$,HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *acetonitrile* and 25 volumes of a 0.17 per cent v/v solution of *glacial acetic acid*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 120 mg of Fexofenadine Hydrochloride, disperse in 20 ml of 0.17 per cent v/v solution of *glacial acetic acid*, with vigorous shaking for 30 minutes and dilute to 100.0 ml with *acetonitrile*, shake vigorously for 60 minutes, filter.

Reference solution (a). A solution containing 0.025 per cent w/v of *fexofenadine hydrochloride IPRS* and 0.005 per cent w/v of *fexofenadine impurity A IPRS* in the solvent mixture. Dilute 3 ml and 4.5 ml, respectively, of the solutions to 50.0 ml with the mobile phase.

Reference solution (b). A 0.025 per cent w/v solution of *fexofenadine hydrochloride IPRS* in the solvent mixture. Dilute 4 ml of the solution to 100.0 ml with the mobile phase. Dilute 6 ml of the solution to 100.0 ml with the mobile phase.

Use the chromatographic system described under Assay.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 6.0 per cent.

Inject reference solution (a). The relative retention time with respect to fexofenadine for fexofenadine impurity A is about 1.6. The resolution between fexofenadine and fexofenadine impurity A is not less than 7, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent and not more than 3.0 per cent for fexofenadine and fexofenadine impurity A, respectively.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of the peak due to fexofenadine impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.4 per cent), the area of any individual impurity is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent), the area of the peak due to decarboxilated degradant [(+)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene having a relative retention time of 6.7 is not more than 0.12 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent) and the sum of all the impurities is not more than 0.5 per cent. Ignore any peak with an area less than 0.04 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *acetonitrile* and 25 volumes of 0.17 per cent v/v *glacial acetic acid* in *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 150 mg of Fexofenadine Hydrochloride, disperse in 20 ml of a 0.17 per cent v/v solution of *glacial acetic acid*, with vigorous shaking for 30 minutes and dilute to 100.0 ml with *acetonitrile*, shake vigorously for 60 minutes and filter. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. A 0.025 per cent w/v solution of *fexofenadine hydrochloride IPRS* in the solvent mixture. Dilute 3 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 64 volumes of a buffer solution prepared by diluting 7.5 ml of *acetonitrile* and 7.5 ml of *triethylamine* to 1000 ml with 0.17 per cent v/v *glacial acetic acid* in *water*, adjusted to pH 5.2 with *orthophosphoric acid* and 36 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{32}H_{39}NO_4$, HCl in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Prolonged-release Tablets

Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Sustained-release Tablets; Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Extended-release Tablets

Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Fexofenadine Hydrochloride and Pseudoephedrine Hydrochloride Prolonged-release Tablets contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amounts of fexofenadine hydrochloride, $C_{32}H_{39}NO_4$, HCl and pseudoephedrine hydrochloride, $C_{10}H_{15}NO$, HCl.

Usual strength. Fexofenadine Hydrochloride, 60 mg and Pseudoephedrine Hydrochloride, 120 mg.

Identification

A. In the Assay, the principal peaks in the chromatogram obtained with test solution (b) correspond to the principal peaks in the chromatogram obtained with reference solution (f).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel G.

Mobile phase. A mixture of 50 volumes of *toluene*, 45 volumes of *ethanol* and 5 volumes of *ammonium hydroxide*.

Test solution. Transfer a quantity of the powdered tablets containing 30 mg of Fexofenadine Hydrochloride to a 10-ml volumetric flask, add 5.0 ml of *methanol* and shake vigorously for 2 minutes, filter.

Reference solution. Dissolve suitable quantities of *fexofenadine hydrochloride IPRS* and *pseudoephedrine hydrochloride IPRS* in *methanol* to obtain a solution having concentration similar to that of the test solution.

Apply to the plate 10 µl of each solutions. After development, dry the plate in air. Heat the plate at 105° until the odour of ammonia disappears. Allow the plate to cool and examine under ultraviolet light at 254 nm. The Rf value of the principal spots in the chromatogram obtained with the test solution correspond to the spots in the chromatogram obtained with the reference solution.

NOTE —The Rf values of fexofenadine and pseudoephedrine are 0.17 and 0.39 respectively.

Tests

Dissolution (2.5.2).

For Fexofenadine hydrochloride —

Apparatus No. 2 (Paddle)

Medium. 900 ml of *0.001M hydrochloric acid,*

Speed and time. 50 rpm, 15 minutes and 45 minutes for fexofenadine hydrochloride and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve suitable quantities of *fexofenadine hydrochloride IPRS* and *pseudoephedrine hydrochloride IPRS* in the dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

NOTE — A small amount of methanol, not more than 0.5 per cent of the total volume, can be used to dissolve fexofenadine hydrochloride IPRS.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with sulphonated fluorocarbon polymer coated on a solid spherical core (strong cation- exchange packing) (10 µm) (Such as partisil 10 SCX),
- mobile phase: a mixture of 45 volumes of a buffer solution prepared by dissolving 7.0 g of *monobasic sodium phosphate* in 1000 ml of *water*, adjusted to pH 2.0 with *orthophosphoric acid* and 55 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to fexofenadine and pseudoephedrine is not less than 3.0, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent, for both the peaks.

Inject the reference solution and the test solution.

Calculate the content of $C_{32}H_{39}NO_4$, HCl and $C_{10}H_{15}NO$, HCl in the medium.

Q. After 15 minutes, not less than 65 per cent and at 45 minutes, not less than 80 per cent of the stated amount of $C_{32}H_{39}NO_4$,HCl.

For Pseudoephedrine hydrochloride — Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14),

Buffer solution. A solution prepared by dissolving 6.8 g of *sodium acetate* and 16.22 g of *sodium octanesulphonate* in 1000 ml of *water,* adjusted to pH 4.6 with *glacial acetic acid.*

Solvent mixture. 60 volumes of *methanol* and 40 volumes of the buffer solution.

Test solution (a). Disperse a sufficient quantity of the intact tablets containing 0.6 g of Fexofenadine Hydrochloride in 300 ml of *methanol,* with the aid of mechanical shaker for 60 minutes. Add 150 ml of the buffer solution and sonicate for 60 minutes at 40°, with intermittent shaking, cool to room temperature and dilute to 500.0 ml with the buffer solution, filter.

Test solution (b). Dilute 2.0 ml of test solution (a) to 50.0 ml with mobile phase.

Reference solution (a). Weigh and transfer 40 mg of *pseudoephedrine hydrochloride IPRS* to a 50-ml volumetric flask, add 5 ml of *tert-butyl hydroperoxide solution,* sonicate to dissolve. Cover the flask opening with aluminium foil and place the flask in an oven at 90° for 60 minutes, Remove from the oven and allow to cool, dilute to volume with the mobile phase (to generate ephedrine impurity).

Reference solution (b). Dissolve 20 mg, each of, *fexofenadine related compound A IPRS* and decarboxylated degradant in 60 ml of *methanol* and dilute to 100.0 ml with the buffer solution. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (c). A 0.048 per cent w/v solution of *fexofenadine hydrochloride IPRS* in the mobile phase.

Reference solution (d). A 0.12 per cent w/v solution of *pseudoephedrine hydrochloride IPRS* in the mobile phase.

Reference solution (e). Dilute 15.0 ml of reference solution (b), 5.0 ml of reference solution (c) and 4.0 ml of reference solution (d) to 50.0 ml with the mobile phase.

Reference solution (f). Dilute suitable volumes of reference solution (c) and reference solution (d) with the mobile phase to obtain a solution having similar concentration to that of test solution (b).

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm, packed with a sulphonated fluorocarbon polymer coated on spherical core (strong cation exchange packing) (5 µm) (Such as Adsorbosphere XL SCX) connected in series to a column 25 cm x 4.6 mm, packed with phenyl group bonded to porous silica (5 µm) (Such as Zorbax SB phenyl),
- column temperature: 35°,
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 6.8 g of *sodium acetate* and 16.22 g of *sodium 1-octanesulfonate* in 1000 ml of *water,* adjusted to pH 4.6 with *glacial acetic acid,* and 65 volumes of *methanol,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set 215 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Fexofenadine	1.0	—
Pseudoephedrine	1.0	—
Ephedrone[a]	1.2	2.54
Fexofenadine related compound A[1b]	1.2	—
Tertiary dehydrated impurity[2b]	1.8	—
Decarboxylated degradant[3b]	3.1	—

[a]Relative retention time with reference to pseudoephedrine.

[b]Relative retention time with reference to fexofenadine.

[1]2-(4{4-[4-(hydroxydephenyl methyl)piperidin-1-yl]butanoxyl]-2-methyl propanoic acid,

[2]4-[4{4-(Diphenylmethylene)-1-piperidinyl}-1-hydroxybutyl]-2, 2-dimethyl phenyl acetic acid,

[3](±)-4-(1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-isopropylbenzene.

Inject reference solution (a) and (e). The test is not valid unless the resolution between the peaks due to pseudoephedrine and ephedrone is not less than 1.7 and the relative standard deviation for replicate injections is not more than 1.0 per cent for pseudoephedrine peak in the chromatogram obtained with reference solution (a), the resolution between the peaks due to fexofenadine and fexofenadine related compound A is not less than 2.0, the relative standard deviation for replicate injections is not more than 1.0 per cent for fexofenadine peak and not more than 3.0 per cent for fexofenadine related compound A and decarboxylated degradant in the chromatogram obtained with reference solution (e).

Inject reference solution (e) and test solution (a). In the chromatogram obtained with test solution (a), the area of any peak corresponding to fexofenadine related compound A is not more than 0.8 times the area of the corresponding peak in the chromatogram obtained with reference solution (e) (0.4 per cent), the area of any peak corresponding to decarboxylated degradant is not more than 0.4 times the area of the corresponding peak in the chromatogram obtained with reference solution (e) (0.2 per cent), the area of any peak corresponding to ephedone is not more than 0.05 times the area of the pseudoephedrine peak in the chromatogram obtained with reference solution (e) (0.2 per cent), the area of any other secondary peak is not more than 0.05 times the area of fexofenadine peak in the chromatogram obtained with reference solution (e) (0.2 per cent). Ignore any peak with an

area less than 0.0125 times the area of fexofenadine peak in the chromatogram obtained with reference solution (e) (0.05 per cent).

The sum of all the impurities is not more than 0.8 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

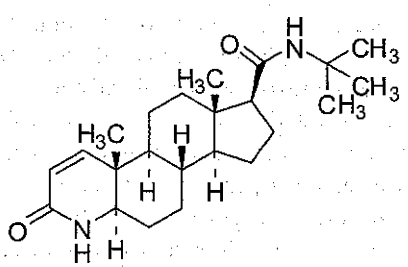
Inject reference solution (a) and (e). The test is not valid unless the resolution between the peaks due to pseudoephedrine and ephedrone is not less than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent for pseudoephedrine peak in the chromatogram obtained with reference solution (a), the resolution between the peaks due to fexofenadine and fexofenadine related compound A is not less than 2.0 and the relative standard deviation for replicate injection is not more than 1.0 per cent for fexofenadine peak in the chromatogram obtained with reference solution (e).

Inject reference solution (f) and test solution (b).

Calculate the content of $C_{32}H_{39}NO_4$, HCl and $C_{10}H_{15}NO$, HCl in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Finasteride



$C_{23}H_{36}N_2O_2$ Mol. Wt. 372.6

Finasteride is 17*β*-(*N-tert*-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one.

Finasteride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{23}H_{36}N_2O_2$, calculated on the dried basis.

Category. Antiandrogen.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *finasteride IPRS* or with the reference spectrum of finasteride.

Tests

Specific optical rotation (2.4.22). +12.0° to +14.0°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture: Equal volumes of *acetonitrile* and *water*.

Test solution (a). Dissolve 25.0 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Test solution (b). Dissolve 100.0 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). Dissolve 25.0 mg of *finasteride IPRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (b). Dissolve 10 mg of *finasteride for peak identification IPRS* (containing impurities A and C) in 1.0 ml of the solvent mixture.

Reference solution (c). Dilute 1.0 ml of test solution (b) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated end capped octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- column temperature: 60°,
- mobile phase: a mixture of 10 volumes *acetonitrile*, 10 volumes of *tetrahydrofuran* and 80 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 15 µl.

Name	Relative retention time	Correction factor
Finasteride impurity A[1]	0.9	2.4
Finasteride	1.0	—
Finasteride impurity C[2]	1.3	0.72

[1] *N*-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androstane-17β-cabroxamide carboxamide (Δ-1,5-aza amide)

[2] *N*-(1,1-dimethylethyl)-3-oxo-4-azaandrosta-1,5-diene-17β-(dihydrofinasteride).

Inject reference solution (b) and (c). The test is not valid unless the signal to noise ratio is not less 40 for the principal peak in the chromatogram obtained with reference solution (c) and the peak to valley ratio is not less than 5.0 where Hp = height above the baseline of the peak due to impurity A and Hv = height above the baseline of the lowest point of the curve separating this peak from the peak due to finasteride in the chromatogram obtained with reference solution (b).

Inject reference solution (c) and test solution (b). Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution (b) the area of secondary peak corresponding to finasteride impurity A and finasteride impurity C is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (c) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent). Ignore any peak with an area less than 0. 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a) and test solution (a).

Calculate the content of $C_{23}H_{36}N_2O_2$.

Storage. Store protected from light.

Finasteride Tablets

Finasteride Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of finasteride, $C_{23}H_{36}N_2O_2$.

Usual strength. 5 mg.

Identification

In the test for Dissolution, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. Dissolve a weighed quantity of *finasteride IPRS* in a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile* and dilute with the same mixture to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 5.0 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 45°,
- mobile phase: a mixture of 42 volumes of *water* and 58 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 200 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{36}N_2O_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{23}H_{36}N_2O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water.*

Test solution. Dissolve a quantity of powdered tablets containing 100 mg of Finasteride in 30 ml of the solvent mixture and dilute to 50.0 ml with the solvent mixture, centrifuge and filter the supernatant liquid.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 2),
- column temperature: 60°,
- mobile phase: a mixture of 10 volumes of *acetonitrile*, 10 volumes of *tetrahydrofuran* and 80 volumes of *water,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Finasteride impurity A[1]	0.9	2.4
Finasteride (Retention time about: 28 minutes)	1.0	—
Finasteride impurity B[2]	1.2	—
Finasteride impurity C[3]	1.3	0.72

[1] *N*-(1,1-dimethylethyl)-3-oxo-4-aza-5a-androstane-17b-carboxamide,
[2] Methyl 3-oxo-4-aza-5a-androst-1-ene-17b-carboxylate,
[3] *N*-(1,1-dimethylethyl)-3-oxo-4-azaandrosta-1,5-diene-17b-carboxamide.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to finasteride impurities A, B and C is not more than 0.3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent). The area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all other secondary peaks is not more than 0.6 times the area of the principal peak in the chromatogram obtained with the reference solution (0.6 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions and reference solution as described under Assay.

Test solution. Transfer one tablet to a suitable volumetric flask, add 5 ml of a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile,* disperse with the aid of ultrasound for 20 minutes. Dilute, as necessary with the solvent mixture with intermittent shaking to produce a solution containing 0.01 per cent w/v of finasteride, mix and filter.

Calculate the content of $C_{23}H_{36}N_2O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of Finasteride with 25 ml of *water*. Add 350 ml of a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile,* mix with the aid of ultrasound for 30 minutes, dilute to 500 ml with *acetonitrile*, centrifuge and filter the supernatant liquid.

Reference solution. A 0.01 per cent w/v solution of *finasteride IPRS* in a mixture of 3 volumes of *water* and 7 volumes of *acetonitrile*.

Chromatographic system

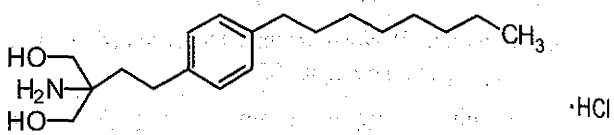
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature: 45°,
- mobile phase: a mixture of equal volumes of *acetonitrile* and *0.0025 M* of *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{36}N_2O_2$ in the tablets.

Fingolimod Hydrochloride



$C_{19}H_{33}NO_2$, HCl Mol Wt. 343.9

Fingolimod Hydrochloride is 2-amino-2-[2-(4-octylphenyl) ethyl] propane-1,3-diol hydrochloride.

Fingolimod Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{33}NO_2$,HCl, calculated on the anhydrous basis.

Category. Immunomodulator

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fingolimod hydrochloride IPRS* or with the reference spectrum of fingolimod hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *water* and 50 volumes of 0.1 per cent w/v solution of *orthophosphoric acid*.

Test solution. Dissolve 60 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). A 0.0003 per cent w/v solution of *fingolimod hydrochloride IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c). A 0.06 per cent w/v solution of *fingolimod hydrochloride system suitability mixture IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- mobile phase: A. 0.1 per cent w/v solution of *orthophosphoric acid* in *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
20	5	95
23	5	95
23.1	80	20
33	80	20

Name	Relative retention time	Correction factor
Fingolimod hexyl homolog[1]	0.82	0.91
Fingolimod heptyl homolog[2]	0.93	---
Fingolimod	1.0	---
Fingolimod nonyl homolog[3]	1.13	---
Fingolimod decyl homolog[4]	1.23	---
3-phenethyl fingolimod analog[5]	1.97	0.77
2-phenethyl fingolimod analog[6]	2.0	0.71

[1]2-amino-2-(4-hexylphenethyl)propane-1,3-diol,

[2]2-amino-2-(4-heptylphenethyl)propane-1,3-diol,

[3]2-amino-2-(4-nonylphenethyl)propane-1,3-diol,

[4]2-amino-2-(4-decylphenethyl)propane-1,3-diol,

[5]2-amino-2-[4-octyl-3-(4-octylphenethyl)phenethyl]propane-1,3-diol,

[6]2-amino-2-[4-octyl-2-(4-octylphenethyl)phenethyl]propane-1,3-diol.

The relative retention time with reference to fingolimod for O-acetyl fingolimod (2-amino-2-(hydroxymethyl)-4-(4-octylphenyl)butyl acetate) is about 1.11.

Inject reference solution (a), (b) and (c). The test is not valid unless the resolution between the peaks due to O-acetyl fingolimod and fingolimod nonyl homolog is not less than 1.2 and peaks due to 2-phenethyl fingolimod analog and 3-phenethyl fingolimod is not less than 0.8 in the chromatogram obtained with reference solution (c), the relative standard deviation for replicate injections is not more than 10 per cent in the chromatogram obtained with reference solution (a) and signal-to-noise ratio is not less than 10 in the chromatogram obtained with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to fingolimod hexyl homolog and fingolimod decyl homolog, each of, is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any peak corresponding to fingolimod heptyl homolog, fingolimod nonyl homolog, 3-phenehyl fingolimod analog and 2-phenethyl finolimd analog, each of, is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.3 g.

Microbial contamination (2.2.9). The total aerobic viable count is not more than 10^3 CFU per g. The total combined molds and yeasts count is not more than 10^2 CFU per g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

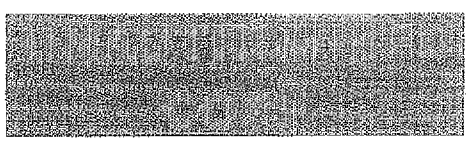
Reference solution. A 0.06 per cent w/v solution of *fingolimod hydrochloride IPRS* in the solvent mixture.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

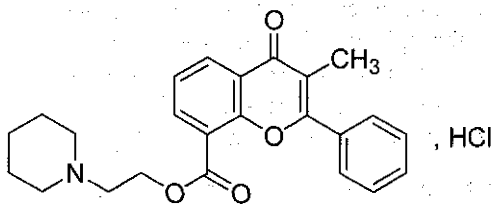
Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{33}NO_2,HCl$.

Storage. Store protected from moisture and at a temperature not exceeding 30°.



Flavoxate Hydrochloride



$C_{24}H_{25}NO_4,HCl$ Mol. Wt.427.9

Flavoxate Hydrochloride is 2-Piperidinoethyl 3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxylate hydrochloride.

Flavoxate Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{24}H_{25}NO_4,HCl$, calculated on the dried basis.

Category. Anticholinergic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flavoxate hydrochloride IPRS* or with the reference spectrum of flavoxate hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Solvent mixture. 20 volumes of a 0.04 per cent w/v solution of *potassium dihydrogen phosphate,* adjusted to pH 3.0 with *orthophosphoric acid* and 80 volumes of *acetonitrile.*

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.006 per cent w/v of *flavoxate impurity A IPRS* and 0.003 per cent w/v of *flavoxate impurity B IPRS* and 0.002 per cent w/v of the substance under examination in the solvent mixture. Dilute 1.0 ml of the solution to 20.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.044 per cent w/v solution of *dipotassium hydrogen phosphate,* adjusted to pH 7.5 with *orthophosphoric acid,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	20	80
10	20	80
20	10	90
25	10	90
28	20	80

Name	Relative retention time
Flavoxate impurity A[1]	0.2
Flavoxate impurity B[2]	0.8
Flavoxate (Retention time: about 10 minutes)	1.0

[1]3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylic acid,

[2]ethyl 3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylate.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to fluvoxate impurity B and flavoxate is not less than 4.0.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to fluvoxate impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent), the area of any peak corresponding to fluvoxate impurity B is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all other secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. *(NOTE — In order to avoid overheating, mix thoroughly throughout and stop the titration immediately after the end-point has been reached).*

Dissolve 0.35 g in 10 ml of *anhydrous formic acid* and add 40 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04279 g of $C_{24}H_{25}NO_4,HCl$.

Storage. Store protected from light.

Flavoxate Tablets

Flavoxate Tablets contain Flavoxate Hydrochloride. The tablets are coated.

Flavoxate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of flavoxate hydrochloride, $C_{24}H_{25}NO_4,HCl$.

Usual strength. 200 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.2 g of Flavoxate Hydrochloride with 10 ml of *dichloromethane*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flavoxate hydrochloride IPRS* or with the reference spectrum of flavoxate hydrochloride.

B. In the test for Related substances, by applying 10 µl of each solution, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No.1 (Basket),

Medium. 900 ml of *0.1 M hydrochloric acid,*

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the medium, if necessary, at the maxima at about 294 nm (2.4.7). Calculate the content of $C_{24}H_{25}NO_4,HCl$ in the medium from the absorbance obtained from a solution of known concentration of *flavoxate hydrochloride IPRS* prepared by dissolving in minimum amount of *methanol* and diluted with the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{24}H_{25}NO_4,HCl$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *18 M ammonia*, 80 volumes of *propan-2-ol* and 200 volumes of *ethyl acetate*.

Test solution (a). Disperse a quantity of the powdered tablets containing 0.2 g of Flavoxate Hydrochloride with 10 ml of *chloroform* and filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 20.0 ml with *chloroform*.

Reference solution (a). A 0.015 per cent w/v solution of *3- methylflavone-8-carboxylic acid ethyl ester IPRS* in *chloroform*.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 500.0 ml with *chloroform*.

Reference solution (c). A 0.1 per cent w/v solution of *flavoxate hydrochloride IPRS* in *chloroform*.

Reference solution (d). A 0.03 per cent w/v solution of *3-methylflavone-8-carboxylic acid IPRS* in *chloroform*.

Apply 10 µl of reference solution (a), (c), (d), test solution (b), 25 µl of reference solution (b) and 50 µl of test solution (a). After removal of the plate, allow it to dry in air and examine under ultraviolet light at 254 nm. Any spot corresponding to 3-methylflavone-8-carboxylic acid ethyl ester in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.15 per cent) and any other secondary spot in the chromatogram obtained with test solution (a), other than the spot corresponding to 3-methyflavone-8-carboxylic acid is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent).

3-Methylflavone-8-carboxylic acid. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 4 volumes of *glacial acetic acid*, 25 volumes of *ethyl acetate* and 70 volumes of *cyclohexane*.

Test solution. Disperse a quantity of the powdered tablets containing 0.2 g of Flavoxate Hydrochloride with 10 ml of *chloroform* and filter.

Reference solution. A 0.01 per cent w/v solution of *3-methylflavone-8-carboxylic acid IPRS* in *chloroform*.

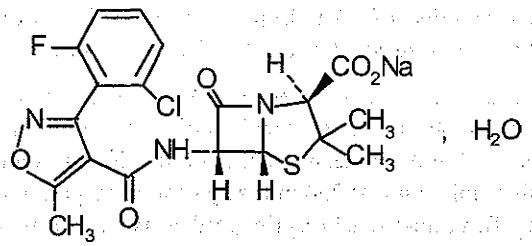
Apply 50 µl of each solution. After removal of the plate, allow it to dry in air and spray with *dilute potassium iodobismuthate solution*. Any spot corresponding to 3-methylflavone-8-carboxylic acid in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of powder containing 1 g of Flavoxate Hydrochloride with 600 ml of *0.1 M hydrochloric acid* with the aid of ultrasound for 10 minutes. Place in a water-bath at 70° for 90 minutes, cool and dilute to 1000 ml with *0.1 M hydrochloric acid*. Dilute 5.0 ml to 250.0 ml with *0.1 M hydrochloric acid* and measure the absorbance at 293 nm (2.4.7). Calculate the content of $C_{24}H_{25}NO_4$,HCl from the absorbance obtained by using a 0.002 per cent w/v solution of *flavoxate hydrochloride IPRS* in *0.1M hydrochloric acid*.

Storage. Store protected from light.

Flucloxacillin Sodium



$C_{19}H_{16}ClFN_3NaO_5S,H_2O$ Mol. Wt. 493.9

Flucloxacillin is sodium (2*S*,5*R*,6*R*)-6-[[[3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4- yl]carbonyl] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2- carboxylate monohydrate.

Flucloxacillin contains not less than 95.0 per cent and not more than 102.0 per cent of $C_{19}H_{16}ClFN_3NaO_5S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white hygroscopic, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and, C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *flucloxacillin sodium IPRS* or with the reference spectrum of flucloxacillin sodium.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

Mobile phase. A mixture of 30 volumes of *acetone* and 70 volumes of 15.4 per cent w/v solution of *ammonium acetate* adjusted to pH 5.0 with *glacial acetic acid*.

Test solution. Dissolve 25 mg of the substance under examination in 5 ml of *water*.

Reference solution (a). A 0.5 per cent w/v solution of *flucloxacillin sodium IPRS* in *water*.

Reference solution (b). A solution containing 0.5 per cent w/v each of *flucloxacillin sodium IPRS*, *cloxacillin sodium IPRS* and *dicloxacillin sodium IPRS* in *water*.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and expose to iodine vapour until the spots appear and examine in day light. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

C. Place 2 mg in a test-tube, moist with 0.05 ml of *water* and add 2 ml of *sulphuric acid-formaldehyde reagent*. Mix the contents of the tube by swirling; the colour of the solution is slightly greenish-yellow. Place the test-tube in a water-bath for 1 minute; the solution becomes yellow.

D. It gives reaction (A) of sodium (2.3.1).

Tests

Solution A. A 10.0 per cent w/v solution in *carbon dioxide-free water*.

Appearance of solution. Solution A is clear (2.4.1) and its absorbance at 430 nm (2.4.7) is not more than 0.04.

pH (2.4.24). 5.0 to 7.0, determined in solution A.

Specific optical rotation (2.4.22). + 158° to + 168°, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). A 0.001 per cent w/v solution of *flucloxacillin sodium IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v each of *flucloxacillin sodium IPRS* and *cloxacillin sodium IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27 per cent w/v solution of

potassium dihydrogen phosphate, adjusted to pH 5.0 with *sodium hydroxide solution*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cloxacillin (1st peak) and flucloxacillin (2nd peak) is not less than 2.5.

Inject reference solution (a) and the test solution. Run the chromatogram 6 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm, determined by method B.

2-Ethylhexanoic acid (2.3.51). Not more than 0.8 per cent.

Water (2.3.43). 3.0 per cent to 4.5 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *flucloxacillin sodium IPRS* in the mobile phase.

Use chromatographic system as described under test for Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{16}ClFN_3NaO_5S$.

Flucloxacillin sodium intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per mg of flucloxacillin sodium.

Storage. Store protected from moisture, at a temperature not exceeding 30°. If it is intended for use in manufacture of parenteral preparation, the container should be sterile, airtight and tamper proof.

Flucloxacillin Capsules

Flucloxacillin Sodium Capsules

Flucloxacillin Capsules contain Flucloxacillin Sodium.

Flucloxacillin Capsules contain not less than 92.5 per cent and not more than 110.0 per cent of the stated amount of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$.

Usual strengths: 250 mg and 500 mg equivalent to flucloxacillin.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flucloxacillin sodium IPRS* or with the reference spectrum of flucloxacillin sodium.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the contents of capsules containing 0.1 g of flucloxacillin with 80 ml of the mobile phase for 15 minutes and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution *(b)*. A solution containing 0.01 per cent w/v each of *flucloxacillin sodium IPRS* and *cloxacillin sodium IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 5.0 with *2 M sodium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to cloxacillin and flucloxacillin is not less than 2.5.

Inject reference solution (a) and the test solution. Run the chromatogram 6 times the retention time of the principal peak. In the chromatogram obtained with test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent). Ignore any peak with an area less than

0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the contents of capsules containing 50 mg of Flucloxacillin in about 40 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 50 ml with the mobile phase. Further dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). A 0.011 per cent w/v solution of *flucloxacillin sodium IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v each of *flucloxacillin sodium IPRS* and *cloxacillin sodium IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cloxacillin (1st peak) and flucloxacillin (2nd peak) is not less than 2.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{19}H_{17}ClFN_3O_5S$ in the capsules.

Labelling. The label states the quantity of the active ingredient in terms of the equivalent amount of flucloxacillin.

Flucloxacillin Oral Solution

Flucloxacillin Oral Solution is a mixture consisting of Flucloxacillin Sodium with buffering agents and other excipients and a suitable flavoring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of *water* just before use.

Flucloxacillin Oral Solution contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of flucloxacillin.

Usual strengths. 125 mg per 5 ml; 250 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 4.0 to 7.0.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately weighed quantity of the oral suspension containing 50 mg of Flucloxacillin to a 50.0 ml volumetric flask, add 40 ml of mobile phase, shake for 15 minutes, dilute to volume with mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). A 0.011 per cent w/v solution of *flucloxacillin sodium IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v each of *flucloxacillin sodium IPRS* and *cloxacillin sodium IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a 0.27 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 5.0 with *2 M sodium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to cloxacillin (first peak) and flucloxacillin (second peak) is not less than 2.5 in the chromatogram obtained with reference solution (b) and in the chromatogram obtained with reference solution (a), the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

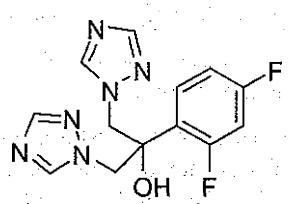
Inject reference solution (a) and the test solution.

Determine the weight per ml of the oral solution (2.4.29) and calculate the content of $C_{19}H_{17}ClFN_3O_5S$, weight in volume.

1 mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

Labelling. The quantity of active ingredient is stated in terms of the equivalent amount of flucloxacillin.

Fluconazole



$C_{13}H_{12}F_2N_6O$ Mol. Wt. 306.3

Fluconazole is 2-(2,4-difluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol.

Fluconazole contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{13}H_{12}F_2N_6O$, calculated on the dried basis.

Category. Antifungal.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluconazole IPRS* or with the reference spectrum of fluconazole.

B. When examined in the range 200 nm to 350 nm (2.4.7), a 0.025 per cent w/v solution in *methanol* shows absorption maxima at about 266 nm and 261 nm.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *methanol* is clear (2.4.1) and colourless (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 300 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution. A 0.003 per cent w/v solution of *fluconazole IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 40°,
- mobile phase: 80 volumes of *water* and 20 volumes of *acetonitrile*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

The relative retention time with reference to fluconazole for 2-(2-fluoro-4-(1*H*-1,2,4-triazol-1-yl)phenyl]-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol (fluconazole impurity A) is about 0.49, for 2-(4-fluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol (fluconazole impurity B) is about 0.81 and for 1',1' - (1,3-phenylene)di(1*H*-1,2,4-triazole) (fluconazole impurity C) is about 0.86.

The correction factor of fluconazole impurity A is 0.08, for fluconazole impurity B is 1.29 and for fluconazole impurity C is 0.05.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of impurity peak at relative retention time about 0.6 is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent), the area of secondary peak due to fluconazole impurity A or fluconazole impurity C is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (0.2 per cent); the area of any peak corresponding to fluconazole impurity B is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (0.1 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

Iron (2.3.14). Dissolve 2.0 g in 20 ml of *ethanol* and 20 ml of *water* and mix; the resulting solution complies with the limit test for iron (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.2 g in 100 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01531 g of $C_{13}H_{12}F_2N_6O$.

Storage. Store protected from moisture.

Fluconazole Capsules

Fluconazole Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluconazole, $C_{13}H_{12}F_2N_6O$.

Usual strengths. 50 mg; 150 mg; 200 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not more than 1.0 µm, rejecting the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *fluconazole IPRS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Q. Not less than 80 per cent of the stated amount of $C_{13}H_{12}F_2N_6O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the powder containing 100 mg of Fluconazole, disperse in 100.0 ml of the mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of *fluconazole IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *water* and 20 volumes of *acetonitrile*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 50 µl.

Name	Relative retention time	Correction factor
Fluconazole impurity A[1]	0.49	0.08
Fluconazole impurity B[2]	0.81	1.29
Fluconazole impurity C[3]	0.86	0.05
Fluconazole	1.0	---

[1]2-(2-fluoro-4-(1*H*-1,2,4-triazol-1-yl)phenyl]-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol,

[2]2-(4-fluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol,

[3]1',1'- (1,3-phenylene)di(1*H*-1,2,4-triazole).

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 1000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram four times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the powder containing 50 mg of Fluconazole, add 70 ml of the mobile phase, sonicate for 15 minutes and dilute to 100.0 ml with the mobile phase and filter.

Reference solution. A 0.05 per cent w/v solution of *fluconazole IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and 40 volumes of *methanol*, adjusted to pH 3.6 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 261 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{12}F_2N_6O$ in the capsules.

Storage. Store protected from moisture.

Fluconazole Oral Suspension

Fluconazole Oral Suspension is a mixture consisting of fluconazole with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of *water*.

Fluconazole Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluconazole, $C_{13}H_{12}F_2N_6O$.

Usual strengths. 50 mg per 5 ml; 200 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

pH (2.4.24). 3.0 to 5.0.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 13 volumes of *acetonitrile* and 87 volumes of *ammonium formate* solution prepared by dissolving 630 mg of *ammonium formate* in 1000.0 ml *water.*

Test solution. Disperse a quantity of suspension containing 300 mg of fluconazole in 40 ml of the solvent mixture, mix with the aid of ultrasound. Dilute the resulting solution to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.03 per cent w/v solution of *fluconazole IPRS*, 0.0003 per cent w/v solution each of *fluconazole impurity B IPRS*, and *fluconazole impurity C IPRS* in the solvent mixture.

Reference solution (b). A 0.0006 per cent w/v solution of *fluconazole IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- mobile phase: A. a 0.063 per cent w/v solution of *ammonium formate* in *water,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	87	13
20	87	13
35	60	40
50	60	40
52	87	13
60	87	13

Name	Relative retention time
Fluconazole impurity A[1]	0.45
Fluconazole isomer[2]	0.51
Fluconazole impurity B[3]	0.71
Fluconazole impurity C[4]	0.78
Fluconazole	1.0

[1] 2-[2-Fluoro-4-(1*H* -1,2,4-triazol-1-yl)phenyl]-1,3-bis(1*H* -1,2,4-triazol-1-yl)-propan-2-ol, this is a process impurity,

[2] 2-(2,4-Difluorophenyl)-1-(1*H* -1,2,4-triazol-1-yl)-3-(4*H* -1,2,4-triazol-4-yl)propan-2-ol, this is a process impurity,

[3] 2-(4-Fluorophenyl)-1,3-bis (1*H* -1,2,4-triazol-1-yl)propan-2-ol, this is a process impurity,

[4] 1, 1'-(1,3-Phenylene) di (1*H* -1,2,4-triazole), this is a process impurity.

Inject reference solution (a). The test is not valid unless the resolution between peaks due to fluconazole impurity B and fluconazole impurity C is not less than 1.5, the resolution between the fluconazole impurity C and fluconazole is not less than 4.0, and the tailing factor for fluconazole peak is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with test solution, the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with the reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 1.5 times the area of principal peak in the chromatogram obtained with the reference solution (b) (0.3 per cent).

Microbial contamination (2.2.9). Total aerobic microbial count is not more than 100 CFU per g and total combined molds and yeasts is not more than 50 CFU per g. 1 g is free from *Escherichia coli*.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determined by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water*.

Test solution. Transfer a quantity of suspension containing 10 mg of fluconazole in 70 ml of the solvent mixture and mix with the aid of ultrasound. Dilute the resulting solution to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.01 per cent w/v solution of *fluconazole IPRS* and 0.0024 per cent w/v solution of *sodium benzoate IPRS* in the solvent mixture.

Reference solution (b). A 0.01 per cent w/v solution of *fluconazole IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *acetonitrile* and 80 volumes of buffer solution prepared by dissolving 2.72 g *potassium dihydrogen orthophosphate* in 1000 ml *water*, adjusted to pH 2.5 with *dilute orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 50 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to fluconazole and sodium benzoate is not less than 5.0, the tailing factor for fluconazole peak is not more than 2.0 and relative standard deviation for replicate injections of fluconazole peak is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Determine the weight per ml (2.4.29) of the oral suspension.

Calculate the content of $C_{13}H_{12}F_2N_6O$.

Storge. Store protected from moisture at a temperature not exceeding 30°.

Fluconazole Tablets

Fluconazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluconazole, $C_{13}H_{12}F_2N_6O$.

Usual strengths. 50 mg; 100 mg; 150 mg; 200 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not more than 1.0 µm, rejecting the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *fluconazole IPRS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Q. Not less than 80 per cent of the stated amount of $C_{13}H_{12}F_2N_6O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the powdered tablets containing 100 mg of Fluconazole, disperse in 100.0 ml of the mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of *fluconazole IPRS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *water* and 20 volumes of *acetonitrile*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 50 µl.

Name	Relative retention time	Correction factor
Fluconazole impurity A[1]	0.49	0.08
Fluconazole impurity B[2]	0.81	1.29
Fluconazole impurity C[3]	0.86	0.05
Fluconazole	1.0	—

[1]2-(2-fluoro-4-(1*H*-1,2,4-triazol-1-yl)phenyl]-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol,

[2]2-(4-fluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)-propan-2-ol,

[3]1',1' - (1,3-phenylene)di(1*H*-1,2,4-triazole).

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 1000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 50 mg of Fluconazole with 70 ml of the mobile phase, sonicate for 15 minutes and dilute to 100.0 ml with the mobile phase, filter.

Reference solution. A 0.05 per cent w/v solution of *fluconazole IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and 40 volumes of *methanol*, adjusted to pH 3.6 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 261 nm,
- injection volume: 20 µl.

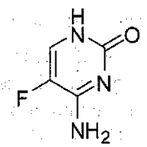
Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{12}F_2N_6O$.

Storage. Store protected from moisture.

Flucytosine



$C_4H_4FN_3O$ Mol. Wt. 129.1

Flucytosine is 4-amino-5-fluoro-2-(1*H*)-pyrimidinone.

Flucytosine contains not less than 98.5 per cent and not more than 101.0 per cent of $C_4H_4FN_3O$, calculated on the dried basis.

Category. Antifungal.

Dose.50 to 150 mg per kg body weight daily, in divided doses every 6 hours.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flucytosine IPRS* or with the reference spectrum of flucytosine.

B. In the test for Related Substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (a).

C. Mix about 5 mg with 45 mg of *heavy magnesium oxide* and ignite in a crucible until an almost white residue is obtained (usually less than 5 minutes). Allow to cool, add 1 ml of *water*, 0.05 ml of *phenolphthalein solution* and about 1 ml of *dilute hydrochloric acid* to render the solution colourless. Filter and add to the filtrate a freshly prepared mixture of 0.1 ml of *alizarin solution* and 0.1 ml of *zirconyl nitrate solution*. Mix, allow to stand for 5 minutes and compare the colour of the solution with that of a blank prepared in the same manner. The colour of the solution changes from red to yellow.

D. To 5 ml of solution A, add 0.15 ml of *bromine water* and shake. The colour of the solution is discharged.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 40 volumes of *water* and 60 volumes of *methanol*.

Mobile phase. A mixture of 1 volume of *anhydrous formic acid*, 15 volumes of *water*, 25 volumes of *methanol* and 60 volumes of *ethyl acetate*.

Test solution (a). Dissolve 50 mg of the substance under examination in 5.0 ml of the solvent mixture.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *flucytosine IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (b) to 100.0 ml with the solvent mixture.

Reference solution (c). Dissolve 5 mg of *fluorouracil IPRS* in 5.0 ml of reference solution (a).

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of cold air, heat at 110° for 10 minutes, place the plate, while hot, in a tank of chlorine vapours for 5 minutes, prepared by adding a mixture of 2 volumes of a 1.5 per cent w/v solution of *potassium permanganate*, 1 volume of *hydrochloric acid* and 1 volume of *water* contained in a beaker placed at the bottom of tank and allow the plate to stand for 15 minutes. Remove the plate from the tank and dry it in a current of cold air until the excess of chlorine is removed and an area of the plate below the line of application gives not more than a faint blue colour with a drop of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and examine the plate in daylight. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Fluoride. Not more than 200 ppm.

NOTE—Prepare and store all solutions in plastic containers.

Buffer solution. Dissolve 58 g of *sodium chloride* in 500 ml of *water*. Add 57 ml of *glacial acetic acid* and 200 ml of a 10.0 per cent w/v solution of *cyclohexylenedinitrilotetra-acetic acid* in *1 M sodium hydroxide*. Adjusted to pH 5.0 to 5.5 with a

20.0 per cent w/v solution of *sodium hydroxide* and dilute to 1000.0 ml with *water*.

Test solution. Dissolve 1.0 g of the substance under examination in *water* and dilute to 100.0 ml with the same solvent.

Reference solution. Dissolve 4.42 g of *sodium fluoride*, previously dried at 120° for 2 hours, in 300 ml of *water* and dilute to 1000.0 ml with the same solvent (0.19 per cent w/v of fluoride). Prepare three reference solutions by diluting 1 ml in 100 ml, 1 ml in 1000 ml and 1 ml in 10000 ml.

To 20.0 ml of each reference solution, add 10.0 ml of the buffer solution and stir with a magnetic stirrer. Introduce the electrodes into the solution and allow to stand for 5 minutes with constant stirring, determining the end point potentiometrically (2.4.25) for fluoride ion, using a fluoride-selective indicator electrode and a silver-silver chloride reference electrode. Determine the potential difference between the electrodes. Plot on semi-logarithmic graph paper the potential difference obtained for each solution as a function of concentration of fluoride. Using exactly the same conditions, determine the potential difference obtained with the test solution and calculate the content of fluoride.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, using platinum crucible.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.1 g, dissolve in 40 ml of *anhydrous acetic acid* and add 100 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01291 g of $C_4H_4FN_3O$.

Storage. Store protected from light.

Flucytosine Capsules

Flucytosine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of flucytosine, $C_4H_4FN_3O$.

Usual strengths. 250 mg; 500 mg.

Identification

A. When examined in the range 200 nm to 400 nm (2.4.7), a 0.002 per cent w/v solution shows absorption maxima at about 260 nm and 350 nm.

B. Shake a portion of the contents of capsules containing about 500 mg of Flucytosine with 10 ml of *water* and filter. To 2 ml of the filtrate, add 1 ml of *sodium pentacyanoaminoferrate reagent* prepared by dissolving 100 mg of *sodium (tri)pentacyanoaminoferrate* in 20 ml of 1 per cent w/v solution of *sodium carbonate solution* and 1 ml of 3 per cent v/v *hydrogen peroxide*; a darker green colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Use the filtrate, dilute if necessary, with the same solvent. Measure the absorbance at the maximum at about 276 nm (2.4.7). Calculate the content of $C_4H_4FN_3O$ in the medium from the absorbance obtained from a solution of known concentration of *flucytosine IPRS*.

Q. Not less than 80 per cent of the stated amount of $C_4H_4FN_3O$.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the contents of 20 capsules containing about 250 mg of Flucytosine, add about 50 ml of *0.1 M hydrochloric acid*, shake for 30 minutes, then dilute with *0.1 M hydrochloric acid* to produce 250.0 ml. Mix, and filter, discard the first 20 ml of the filtrate and dilute 10.0 ml of the filtrate to 250.0 ml with *0.1 M hydrochloric acid*. Further dilute 10.0 ml of the solution to 50.0 ml. with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7). Calculate the content of $C_4H_4FN_3O$ from the absorbance obtained with 0.0008 per cent w/v solution of *flucytosine IPRS* in the same medium.

Storage. Store protected from light.

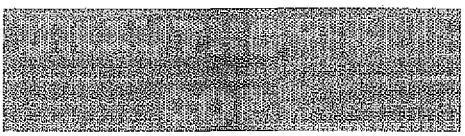
Flucytosine Oral Suspension

Flucytosine Oral Suspension is a suspension of Flucytosine in a suitable flavoured vehicle.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Flucytosine Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of flucytosine, $C_4H_4FN_3O$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of flucytosine $C_4H_4FN_3O$.



Usual strength. 10 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.0 to 5.0.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the oral suspension containing about 5 mg of Flucytosine with 100.0 ml of the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *flucytosine IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of buffer solution prepared by dissolving 1 g of *ammonium acetate* and 1.0 ml of *di-isopropylamine* in 1000 ml of *water*, adjusted to pH 7.5 with *glacial acetic acid* ,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_4H_4FN_3O$ in the suspension.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of $C_4H_4FN_3O$, weight in volume.

Storage. Store protected from light and moisture.

Labelling. The label states (1) the quantity of flucytosine per ml; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

Flucytosine Tablets

Flucytosine Tablets contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of flucytosine, $C_4H_4FN_3O$.

Usual strengths. 250 mg; 500 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.5 g of flucytosine with 100 ml of *methanol* for 30 minutes, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flucytosine IPRS* or with the reference spectrum of flucytosine.

B. When examined in the range 230 to 350 nm (2.4.7), the solution obtained in the Assay shows absorption maxima only at about 286 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance at the maximum at about 276 nm (2.4.7). Calculate the content of $C_4H_4FN_3O$ in the medium from the absorbance obtained from a solution of known concentration of *flucytosine IPRS*.

Q. Not less than 80 per cent of the stated amount of $C_4H_4FN_3O$.

Related substances. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 1 volume of *anhydrous formic acid* , 15 volumes of *water*, 25 volumes of *methanol* and 60 volumes of *ethyl acetate*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Flucytosine with 10.0 ml of a mixture of equal volumes of *13.5 M ammonia* and *methanol*, filter.

Reference solution (a). Dilute 1.0 ml of test solution to 10.0 ml with *methanol (60 per cent)*. Dilute 1.0 ml of the solution to 100.0 ml with *methanol (60 per cent)*.

Reference solution (b). Dilute 1.0 ml of test solution to 10.0 ml and dissolve 5 mg of *fluorouracil IPRS* in 5.0 ml of the resulting solution.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of cold air, heat at 110° for 10 minutes, place the plate, while hot, in a tank of chlorine gas prepared by adding *hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 15 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of *potassium iodide* in *starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide* and *starch solution* and examine the

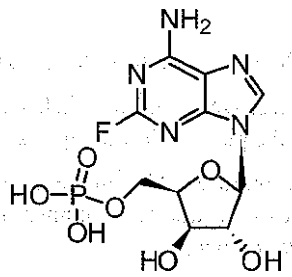
plate in daylight. Any secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

Other tests. Comply with the tests stated under tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of Flucytosine add 80 ml of *0.1 M hydrochloric acid*, shake for 15 minutes and Dilute to 100 .0 ml with *0.1 M hydrochloric acid* and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid*. Further dilute 10.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 286 nm (2.4.7). Calculate the content of $C_4H_4FN_3O$ taking 709 as the specific absorbance at 286 nm.

Storage. Store protected from light.

Fludarabine Phosphate



$C_{10}H_{13}FN_5O_7P$ Mol. Wt. 365.2

Fludarabine Phosphate is [(2*R*,3*R*,4*S*,5*R*)-5-(6-Amino-2-fluoro-9-purinyl)-3,4-dihydroxy-2-oxolanyl]methoxyphosphonic acid.

Fludarabine Phosphate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{10}H_{13}FN_5O_7P$, calculated on the anhydrous and solvent free basis.

CAUTION: Fludarabine Phosphate is cytotoxic; Extra care required to prevent inhaling and exposing the skin to it.

Category. Antineoplastic.

Description. A white to off-white crystalline hygroscopic powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fludarabine phosphate IPRS* or with the reference spectrum of fludarabine phosphate.

Tests

Specific optical rotation (2.4.22). +10° to +14°, determined in a 0.5 per cent w/v solution.

Related substances. A. *For impurities eluting before fludarabine* —

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A 0.002 per cent w/v solution of *fludarabine phosphate IPRS* in the mobile phase.

Reference solution (b). Dissolve 10 mg of the substance under examination in 10 ml of *0.1M hydrochloric acid*. Heat the solution at 80° in a water-bath for 15 minutes

Use chromatographic system as described under Assay.

Name	Relative retention time	Correction factor
Iso-ara-guanine-monophosphate[1]	0.26	4.0
Isoguanine[2]	0.34	2.5
3',5'-Diphosphate analog[3]	0.42	1.9
Fludarabine phosphate	1.0	—

[1]6-Amino-9-β-D-arabinofuranosyl-2-oxo-1*H*-purine 5'-(dihydrogen phosphate)

[2]6-Amino-1*H*-purin-2(9*H*)-one

[3]9-β-D-arabinofuranosyl-2-fluoroadenine3'5'-bis(dihydrogen phosphate)

Inject reference solution (a) and (b). The test is not valid unless the resolution between the iso-ara-guanine monophosphate peak and the isoguanine peak is not less than 2.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to iso-ara-guanine-monophosphate is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent), the area of the peak due to isoguanine is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of the peak due to 3',5'-diphosphate analog is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent) and the area of the any other secondary peak that eluting before fludarabine phosphate is not more than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

B. *For impurities eluting after fludarabine* —

Determine by liquid chromatography (2.4.14), as described under test A with the following modifications.

- mobile phase: a mixture of 80 volumes of *10 mM monobasic potassium phosphate* and 20 volumes of *methanol*,

Name	Relative retention time	Correction factor
Fludarabine phosphate	1.0	—
2-Fluoroadenine[1]	1.5	0.5
2-Fluoro-ara-adenine[2]	1.9	0.6
2-Ethoxyphosphate analog[3]	2.5	1.8

[1] 2-fluoro-9*H*-purin-6-amine

[2] 9-β-D-Arabinofuranosyl-2-fluoroadenine

[3] 2-Ethoxy-9- β-D-arabinofuranosyladenine 5'-(dihydrogen phosphate)

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-fluoroadenine is not more than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any peak corresponding to 2-fluoro-ara-adenine is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any peak corresponding to 2-ethoxyphosphate analog is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

The sum of other secondary peaks obtained in test A and B is not more than 0.5 per cent and the sum of all the secondary peaks obtained in test A and B is not more than 1.5 per cent.

Chlorides (2.3.12). Dissolve 0.125 g in 15 ml of *water*. The solution complies with the limit test for chlorides (0.2 per cent).

Ethanol. Not more than 1.0 per cent.

Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.5 g of the substance under examination in *dimethylformamide* and dilute to 10.0 ml with *dimethylformamide*.

Reference solution. A 0.05 per cent w/v solution of *ethanol* in *dimethylformamide*.

Chromatographic system

- a capillary column 30 m x 0.25 mm, coated with 6 per cent cyanopropylphenyl- 94 per cent dimethylpolysiloxane (film thickness 1.4 µm),
- temperature:
  column. 40° for 10 minutes, then raised at the rate of 5° per minute to 70°, followed by an increase at a rate of 30° per minute to 220°,
- inlet port at 160° and detector at 250°,
- flame ionization detector,
- linear velocity: 27 cm per second, using nitrogen as the carrier gas.

Inject the reference solution. The test is not valid unless and the relative standard deviation for replicate injections is not more than 4.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of C_2H_5OH.

Free phosphate content. Not more than 0.1 per cent.

Test solution. Dissolve 10 mg of Fludarabine Phosphate in 2.0 ml of *water*, heat gently in a test-tube.

Reference solution. A 0.072 per cent w/v solution of *potassium dihydrogen phosphate* in *water*. Dilute 1.0 ml of the solution to 100 ml with *water*.

Molybdovanadic reagent. In a 150-ml beaker, mix 4 g of finely powdered *ammonium molybdate* and 0.1 g of finely powdered *ammonium vanadate*. Add 70 ml of *water* and grind the particles using a glass rod. A clear solution is obtained within a few minutes. Add 20 ml of *nitric acid* and dilute to 100.0 ml with *water*.

In 2.0 ml of the reference solution and the test solution in test tubes, add 2.0 ml *molybdovanadic reagent*. The yellow coloration of the test solution is not more intense than that of the reference solution.

Sodium. Determine by flame photometry (2.4.4).

Test solution. Dissolve 50 mg of the substance under examination in *water* and dilute to 100 ml with *water*.

Reference solution. Dissolve a quantity of *sodium chloride*, previously dried at 105° for 2 hours in *water* and dilute to 250.0 ml with *water* to obtain a solution having a concentration of 2.54 mg of sodium chloride per ml. Dilute the solution with *water* to obtain a solution containing 0.0001 per cent w/v of sodium.

Determine the atomic emission of the solutions at the sodium emission line at 589.0 nm. The emission response obtained with the test solution is not more than the reference solution (0.2 per cent).

Microbial contamination (2.2.9). The total aerobic microbial count is not more than 1000 CFU per g.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 250.0 ml with the mobile phase.

Reference solution. A 0.002 per cent w/v solution of *fludarabine phosphate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 94 volumes of *10 mM monobasic potassium phosphate* and 6 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{13}FN_5O_7P$.

Storage. Store protected from light and moisture, at a temperature between 2° to 8°.

Fludarabine Phosphate Injection

Fludarabine Phosphate Injection is a sterile solution of Fludarabine Phosphate in Water for Injections.

Fludarabine Phosphate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fludarabine phosphate, $C_{10}H_{13}FN_5O_7P$.

CAUTION: Fludarabine Phosphate is cytotoxic; Extra care required to prevent inhaling and exposing the skin to it.

Usual strength. 25 mg per ml.

Identification

A. A 0.0027 per cent w/v solution in *0.1 M hydrochloric acid* gives absorption maximum at 254 nm (2.4.7).

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.0 to 7.1.

Related substances. A. *For impurities eluting before fludarabine—*

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of Fludarabine Phosphate in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A 0.002 per cent w/v solution of *fludarabine phosphate IPRS* in the mobile phase.

Reference solution (b). Dissolve 10 mg of Fludarabine Phosphate in 10 ml of *0.1 M hydrochloric acid.* Heat the solution at 80° in a water-bath for 15 minutes.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 94 volumes of *10 mM monobasic potassium phosphate* in *water* and 6 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Iso-ara-guanine-monophosphate[1]	0.26	4.0
Isoguanine[2]	0.34	2.5
3',5'-Diphosphate analog[3*]	0.42	—
Fludarabine phosphate	1.0	—

*process impurity included for identification only and to be controlled in drug substance.

[1]6-Amino-9-β-D-arabinofuranosyl-2-oxo-1*H*-purine 5'-(dihydrogen phosphate),

[2]6-Amino-1*H*-purin-2(9*H*)-one,

[3]9-β-D-arabinofuranosyl-2-fluoroadenine3'5'-bis(dihydrogen phosphate).

Inject reference solution (a) and (b). The test is not valid unless the resolution between the iso-ara-guanine monophosphate peak and the isoguanine peak is not less than 2.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to iso-ara-guanine-monophosphate is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of the peak due to isoguanine is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of the any other secondary peak that eluting before to fludarabine phosphate is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

B. For impurities eluting after fludarabine —

Determine by liquid chromatography (2.4.14), as described in test A with the following modifications.

- mobile phase: a mixture of 80 volumes of *10 mM monobasic potassium phosphate* in *water* and 20 volumes of *methanol*,

Name	Relative retention time	Correction factor
Fludarabine phosphate	1.0	—
2-Fluoroadenine[1]	1.5	0.5
2-Fluoro-ara-adenine[2]	1.9	0.6
2-Ethoxyphosphate analog[3]*	2.5	—

*process impurity included for identification only and to be controlled in drug substance.

[1]2-fluoro-9*H*-purin-6-amin,

[2]9-β-D-Arabinofuranosyl-2-fluoroadenine,

[3]2-Ethoxy-9- β-D-arabinofuranosyladenine 5'-(dihydrogren phosphate).

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to 2-fluoroadenine is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of the peak due to 2-fluoro-ara-adenine is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of the any other secondary peak that elutes after fludarabine phosphate is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The sum of areas of all the secondary peaks found in test A and B is not more than 2.0 per cent.

Bacterial endotoxins (2.2.3). Not more than 7.7 Endotoxin Units per mg of Fludarabine Phosphate.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Solution A. A 0.69 per cent w/v solution of *monobasic sodium phosphate monohydrate* in *water*, adjusted to pH 4.5 with *1 M sodium hydroxide*.

Test solution. Dilute a volume of injection containing 10 mg of Fludarabine Phosphate in 100.0 ml of solution A.

Reference solution. A 0.01 per cent w/v solution of *fludarabine phosphate IPRS* in solution A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 94 volumes of solution A and 6 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

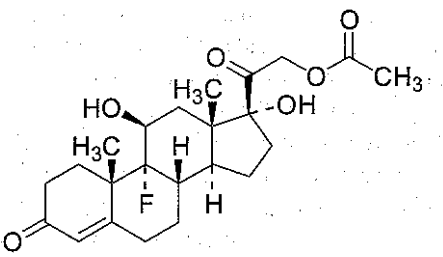
Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{13}FN_5O_7P$ in the injection.

Storage. Store protected from moisture and light, preferably of type I glass at a temperature 2° to 8°.

Fludrocortisone Acetate



$C_{23}H_{31}FO_6$ Mol. Wt. 422.5

Fludrocortisone Acetate is 9α-fluoro-11β,17α,21-trihydroxypregn-4-ene-3,20-dione 21-acetate.

Fludrocortisone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{23}H_{31}FO_6$, calculated on the dried basis.

Category. Mineralocorticoid.

Description. A white or almost white, crystalline powder; hygroscopic.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fludrocortisone acetate IPRS* or with the reference spectrum of fludrocortisone acetate.

B. To a warm 1 per cent w/v solution in *methanol* add an equal volume of *potassium cupri-tartrate solution*; a red precipitate is produced.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *fludrocortisone acetate IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Specific optical rotation (2.4.22). +148° to +156°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol* at the maximum at about 240 nm, 0.39 to 0.42.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve 2 mg of *fludrocortisone acetate IPRS* and 2 mg of *hydrocortisone acetate IPRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *tetrahydrofuran* and 65 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Inject reference solution (a). The retention times are: hydrocortisone acetate, about 8.5 minutes and fludrocortisone acetate about 10 minutes. The test is not valid unless the resolution between the peaks corresponding to hydrocortisone acetate and fludrocortisone acetate is at least 1.0. If this is not achieved, adjust the concentration of tetrahydrofuran in the mobile phase. Increasing the concentration of tetrahydrofuran reduces the retention times.

Inject reference solution (b) and the test solution. Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all such peaks is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.025 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 10 mg, dissolve in 50 ml of *ethanol* and add sufficient *ethanol* to produce 100.0 ml. Dilute 5.0 ml of the solution to 50.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 238 nm. Calculate the content of $C_{23}H_{31}FO_6$ taking 405 as the specific absorbance at 238 nm.

Storage. Store protected from light at a temperature not exceeding 30°.

Fludrocortisone Tablets

Fludrocortisone Acetate Tablets

Fludrocortisone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fludrocortisone acetate, $C_{23}H_{31}FO_6$.

Usual strength. 100 µg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

Test solution. Shake a quantity of the powdered tablets containing 1 mg of Fludrocortisone Acetate with 20 ml of *chloroform* for 5 minutes, filter and evaporate the filtrate to dryness. Dissolve the residue in 4 ml of a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Reference solution (a). Dissolve 25 mg of *fludrocortisone acetate IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.01M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *fludrocortisone acetate IPRS* in minimum amount of *acetonitrile* and dilute with the dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{31}FO_6$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{23}H_{31}FO_6$.

Uniformity of content. Complies with the test stated under Tablets.

Powder one tablet, disperse in 10 ml of *water* and extract with three quantities, each of 5 ml, of *chloroform*. Filter the extracts through a plug of cotton wool moistened with *chloroform*. Evaporate the chloroform on a water-bath just to dryness. Cool and dissolve the residue in 10.0 ml of *ethanol*. Measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{23}H_{31}FO_6$ taking 405 as the specific absorbance at 240 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.5 mg of Fludrocortisone Acetate with 2 ml of *water* for one minute, add 8 ml of *acetonitrile* and shake on a mechanical shaker for 40 minutes. Dilute the mixture to 20.0 ml with *acetonitrile*, centrifuge and use the supernatant liquid.

Test solution (b). Prepare in the same manner as test solution (a) but using 4.0 ml of a solution containing 0.01 per cent w/v of *norethisterone IPRS* (internal standard) in *acetonitrile* and 4.0 ml of *acetonitrile* in place of 8 ml of *acetonitrile*.

Reference solution. Mix 20.0 ml of internal standard, 25.0 ml of a 0.01 per cent w/v solution of *fludrocortisone acetate IPRS* in *acetonitrile* and 10 ml of *water* and dilute to 100.0 ml with *acetonitrile*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (Such as Spherisorb ODS 1),

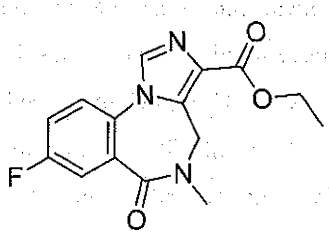
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution and test solution (b).

Calculate the content of $C_{23}H_{31}FO_6$ in the tablets.

Storage. Store protected from light.

Flumazenil



$C_{15}H_{14}FN_3O_3$ Mol. Wt. 303.3

Flumazenil is 4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid, 8-fluoro-5,6-dihydro-5-methyl-6-oxo-, ethyl ester.

Flumazenil contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{14}FN_3O_3$, calculated on the dried basis.

Category. Benzodiazepine toxcity antidote.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flumazenil IPRS* or with the reference spectrum of flumazenil.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

N,N-dimethylformamide diethyl acetal. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *chloroform*, 15 volumes of *glacial acetic acid*, 7.5 volumes of *ethanol* and 2.5 volumes of *water*.

Solvent mixture. Equal volumes of *ethanol* and *chloroform*.

Test solution. Disperse 250 mg of the substance under examination with the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.05 per cent w/v of *flumazenil IPRS* and 0.6 µl per ml of *flumazenil impurity C IPRS (N,N-dimethylformamide diethyl acetal IPRS)* in the solvent mixture.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in cold air and examine under ultraviolet light at 254 nm. Spray the plate with *ninhydrin solution* and heat at 105° for 15 minutes. Any spot at an Rf value 0.04 corresponding to flumazenil impurity C in the chromatogram obtained from the test solution is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay with the following modifications.

Reference solution (c). A 0.0001 per cent w/v solution of *flumazenil IPRS* in the mobile phase.

Name	Relative retention time	Correction factor
Flumazenil impurity A[1]	0.4	0.91
Flumazenil impurity C[2]	0.5	0.67
Flumazenil impurity D[3]	0.7	0.77
Flumazenil impurity B[4]	0.8	0.91
Flumazenil	1.0	—
Flumazenil impurity E[5]	2.2	0.91

[1]8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid,

[2]7-fluoro-4-methyl-3,4-dihydro-2,5*H*-1,4-benzodiazepine-2,5-dione,

[3]ethyl 5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5- *a*][1,4] benzodiazepine-3-carboxylate,

[4]ethyl 8-hydroxy-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-][1,4] benzodiazepine-3-carboxylate,

[5]ethyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5-*a*][1,4] benzodiazepine-3-carboxylate.

Inject reference solution (c) and the test solution. Run the chromatograms 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to flumazenil impurities A, B, C, D and E is not more than twice the area of the principal peak in the chromatogram obatined with reference solution (c) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of

the principal peak in the chromatogram obatined with reference solution (c) (0.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Bacterial endotoxins (2.2.3). Not more than 25.0 Endotoxin Units per mg of flumazenil.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse 25 mg of the substance under examination with the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *flumazenil IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.00064 per cent w/v each of *flumazenil IPRS* and *flumazenil impurity B IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of 800 ml of *water*, adjusted to pH 2.0 with *orthophosphoric acid*, 13 volumes of *methanol* and 7 volumes of *tetrahydrofuran*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 5 µl.

Inject reference solution (a) and (b). The test is not valid the resolution between the peak due to flumazenil impurity B and flumazenil is not less than 4.0, the column efficiency is not less than 1500 theoretical plates for the principal peak and the tailing factor is not more than 1.5 for the principal peak in the chromatogram obtained with reference solution (b) and in the chromatogram obtained with reference solution (a), the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{14}FN_3O_3$.

Storage. Store protected from moisture.

Flumazenil Injection

Flumazenil Injection is a sterile solution of Flumazenil.

Flumazenil Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of $C_{15}H_{14}FN_3O_3$.

Usual strength. 0.1 mg per ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *ethyl acetate* and 10 volumes of *methanol*.

Test solution. Dilute a volume of injection containing 10 mg of Flumazenil to 100.0 ml with *water*.

Reference solution. A 0.01 per cent w/v solution of *flumazenil IPRS* in a mixture of 9 volumes of *water* and 1 volume of *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.4 to 4.6.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay with the following modifications.

Name	Relative retention time	Coprrection factor
Flumazenil impurity A[1]	0.71	1.1
Flumazenil impurity B[2]	0.85	—
Flumazenil	1.0	—

[1]8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid,

[2]ethyl 8-hydroxy-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol-[1,5-*a*][1,4]benzodiazepine-3-carboxylate.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to flumazenil impurity A is not more than 1.0 per cent and the area of any other secondary peak is not more than 0.5 per cent. The sum of areas of all the secondary peaks other than flumazenil impurity A is not more than 1.0 per cent and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization.

Bacterial endotoxins (2.2.3). Not more than 100 Endotoxin Units per mg of flumazenil.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *water*, 20 volumes of *tetrahydrofuran* and 5 volumes of *methanol*.

Test solution. Dilute a volume of injection to obtain a 0.01 per cent w/v solution of Flumazenil in the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *flumazenil IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of 0.02 M phosphate buffer prepared by adding *0.02 M orthophosphoric acid* to *0.02 M monobasic potassium phosphate* to obtain a solution pH 2.7, 20 volumes of *tetrahydrofuran* and 5 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

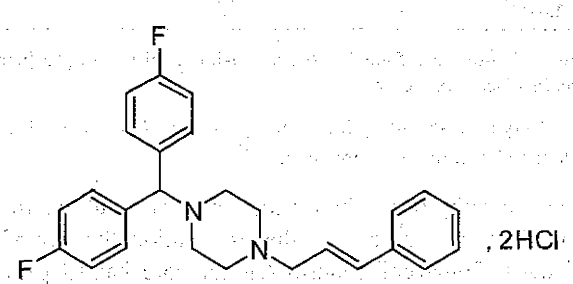
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{14}FN_3O_3$ in the injection.

Storage. Store protected from moisture in single-dose containers, preferably of Type I glass and at controlled room temperature.

Flunarizine Dihydrochloride



$C_{26}H_{28}Cl_2F_2N_2$ Mol. Wt. 477.4

Flunarizine Dihydrochloride is 1-[Bis(4-fluorophenyl)methyl]-4-[(2E)-3-phenylprop-2-enyl]piperazine dihydrochloride.

Flunarizine Dihydrochloride contains not less than 99.0 per cent and not more than 101.5 per cent of $C_{26}H_{28}Cl_2F_2N_2$ calculated on the dried basis.

Category. Antimigraine, Anti-vertigo.

Description. A white or almost white powder, hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flunarizine dihydrochloride IPRS* or with the reference spectrum of flunarizine dihydrochloride.

B. Dissolve 25 mg in 2 ml of *methanol* and add 0.5 ml of *water*, it gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use and protect from light.

Test solution. Dissolve 100 mg of the substance under examination in *methanol* and dilute to 10.0 ml with the same solvent.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 20.0 ml with the same solvent.

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a mixture of 2.38 per cent w/v solution of *tetrabutylammonium hydrogen sulphate* and 0.7 per cent w/v solution of *ammonium acetate*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
12	40	60
15	40	60
16	80	20
20	80	20

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than twice the area of the principal

peak in the chromatogram obtained with the reference solution (0.5 per cent), the area of not more than one other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.25 per cent), the area of any other secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than the 4 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, using platinum crucible.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Dissolve 0.2 g of the substance under examination in 70 ml of *ethanol*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Read the volume added at the second point of inflexion. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02387 g of $C_{26}H_{28}Cl_2F_2N_2$.

Storage. Store protected from light and moisture.

Flunarizine Tablets

Flunarizine Dihydrochloride Tablets

Flunarizine Tablets contain flunarizine dihydrochloride equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of flunarizine, $C_{26}H_{26}F_2N_2$.

Usual strengths. 5 mg; 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the medium if necessary, at the maximum at about 253 nm (2.4.7). Calculate the content of $C_{26}H_{26}F_2N_2$ in the medium from the absorbance obtained from a solution of known concentration of *flunarizine dihydrochloride IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{26}H_{26}F_2N_2$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay with the following modification.

Test solution. Disperse one intact tablet in 5 ml of *water* with the aid of ultrasound, until tablet is completely dispersed and dilute with *methanol* to obtain a solution containing 0.001 per cent w/v solution of Flunarizine.

Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{26}F_2N_2$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powdered tablets containing 20 mg of Flunarizine in 5 ml of *water* with the aid of ultrasound. Add 70 ml of *methanol* and further sonicate to dissolve, dilute to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 100.0 ml with *methanol*.

Reference solution. A 0.0012 per cent w/v solution of *flunarizine dihydrochloride IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 12.5 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Lichrospher 100 RP 18-E),
- mobile phase: a mixture of 20 volumes of a buffer solution prepared by dissolving 1.13 g of *1- hexane sulphonic acid sodium salt (anhydrous)* in 200 ml of *water* and 80 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

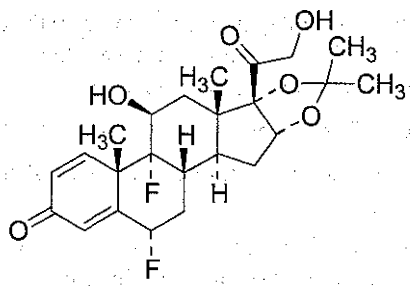
Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{26}F_2N_2$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of flunarizine.

Fluocinolone Acetonide



$C_{24}H_{30}F_2O_6$ Mol. Wt. 452.5

Fluocinolone Acetonide is 6α,9α-difluoro-11β,21-dihydroxy-16α,17α -isopropylidenedioxypregna-1,4-diene-3,20-dione.

Fluocinolone Acetonide contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{24}H_{30}F_2O_6$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if tests A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluocinolone acetonide IPRS* or with the reference spectrum of fluocinolone acetonide.

B. In the related substances test, the principlal peak in the chromatogram obtained with reference solution (b) is similar in retention time to the peak due to *fluocinolone acetonide IPRS* in the chromatogram obtained with reference solution (a).

C. Heat 0.5 ml of *chromic-sulphuric acid* in a test-tube (5 cm x about 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

Tests

Specific optical rotation (2.4.22). +92.0° to +96.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption. Dissolve 15 mg in about 50 ml of *ethanol* and dilute to 100.0 ml with *ethanol*. Dilute 10.0 ml of the solution to 100.0 ml with *ethanol*. Absorbance of the resulting solution at the maximum at about 239 nm, 0.52 to 0.56 (2.4.7).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in *acetonitrile* and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 2.5 mg of *fluocinolone acetonide IPRS* and 2.5 mg of *triamcinolone acetonide IPRS* in 45 ml of *acetonitrile* and dilute to 100 ml with *water*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 450 ml of *acetonitrile* and 500 ml of *water*, allowed to equilibrate, the volume adjusted to 1000 ml with *water* and mixed again,
- flow rate: 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 20 µl.

Inject reference solution (a). The retention times are: triamcinolone acetonide about 8.5 minutes and fluocinolone acetonide about 10 minutes. The test is not valid unless the resolution between the peaks corresponding to triamcinolone acetonide and fluocinolone acetonide is not less than 3.0.

Inject reference solution (b) and the test solution. Continue the chromatography for 4 times the retention time of fluocinolone acetonide. In the chromatogram obtained with the test solution the area of any peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent); not more than one such peak is more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 50 mg in *ethanol*, add sufficient *ethanol* to produce 50.0 ml and mix. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol*. Measure the absorbance of the resulting solution at the maximum at about 238 nm. Calculate the content of $C_{24}H_{30}F_2O_6$, taking 355 as the specific absorbance at 238 nm.

Storage. Store protected from light.

Fluocinolone Cream

Fluocinolone Acetonide Cream

Fluocinolone Cream contains Fluocinolone Acetonide in a suitable cream base.

Fluocinolone Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluocinolone acetonide, $C_{24}H_{30}F_2O_6$.

Usual strengths. 0.0025 per cent; 0.00625 per cent; 0.01 per cent; 0.025 per cent; 0.2 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *n-hexane*, 40 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *triethylamine*.

Test solution. Disperse, by shaking a quantity of the cream containing 0.25 mg of Fluocinolone Acetonide in 2 ml of *chloroform*, add 10 ml of *methanol*, shake vigorously, cool in ice for 15 minutes, centrifuge at 3000 rpm for 15 minutes, decant the clear supernatant liquid, evaporate to dryness on a water-bath in a current of nitrogen and dissolve the residue in 1 ml of *chloroform*.

Reference solution. A 0.025 per cent w/v solution of *fluocinolone acetonide IPRS* in *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, heat at 105° for 5 minutes and spray whilst hot with *blue tetrazolium solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution (a) has the same retention time as that of the peak due to Fluocinolone Acetonide in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

For creams containing 0.025 per cent to 0.2 per cent w/w of fluocinolone acetonide—

Test solution (a). Weigh a quantity of the cream containing about 2.5 mg of Fluocinolone Acetonide, add 60 ml of a solution prepared by adding 80 ml of *methanol* to 20 ml of a 25 per cent w/v solution of *lithium chloride* and disperse by shaking vigorously. Add 100 ml of *cyclohexane*, shake gently for 2 minutes and separate the lower, aqueous methanolic layer, taking care to exclude any solid matter that separates at the interface. Repeat the extraction using a further 25 ml of the *lithium chloride solution*. To the combined extracts add a solution containing 11 g of *alum* in 214 ml of *water* followed by 50 ml of *chloroform*, shake vigorously for about 3 minutes, allow the layers to separate and filter the chloroform extract through filter paper (such as Whatman No 1), previously moistened with *chloroform*, again excluding any solid matter at the interface. Repeat the extraction with 50 ml and 10 ml quantities of *chloroform*, filtering the extracts as before. Evaporate the combined extracts to dryness on a water-bath in a current of nitrogen, dissolve the residue in 5 ml of *chloroform*, transfer to a 10-ml volumetric flask with the aid of *chloroform* and add sufficient *chloroform* to produce 10.0 ml.

Test solution (b). Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.05 per cent w/v solution of *phenacetin* (internal standard) to the chloroform solution before dilution to 10.0 ml.

Reference solution. A solution containing 0.025 per cent w/v of *fluocinolone acetonide IPRS* and 0.005 per cent w/v of *phenacetin* in *chloroform*.

For creams containing 0.01 per cent w/w of fluocinolone acetonide—

Test solution (a). Prepare as described above but using a quantity of the cream containing about 1 mg of Fluocinolone Acetonide.

Test solution (b). Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.02 per cent w/v solution of *phenacetin* (internal standard) to the chloroform solution before diluting to 10.0 ml.

Reference solution. A solution containing 0.01 per cent w/v of *fluocinolone acetonide IPRS* and 0.002 per cent w/v of *phenacetin* in *chloroform*.

For creams containing 0.00625 per cent w/w of fluocinolone acetonide—

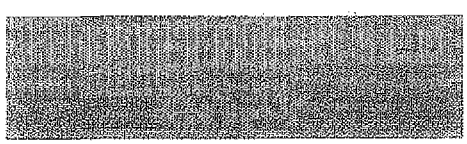
Test solution (a). Prepare as described above but using a quantity of the cream containing about 0.625 mg of Fluocinolone Acetonide.

Test solution (b). Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.0125 per cent w/v solution of *phenacetin* (internal standard) to the chloroform solution before diluting to 10.0 ml.

Reference solution. A solution containing 0.00625 per cent w/v of *fluocinolone acetonide IPRS* and 0.00125 per cent w/v of *phenacetin* in *chloroform*.

For creams containing 0.0025 per cent w/w of fluocinolone acetonide—

Test solution (a). Prepare as described above but using a quantity of the cream containing about 0.25 mg of Fluocinolone Acetonide.



Test solution (b). Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.005 per cent w/v solution of *phenacetin* (internal standard) to the chloroform solution before diluting to 10.0 ml.

Reference solution. A solution containing 0.0025 per cent w/v of *fluocinolone acetonide IPRS* and 0.0005 per cent w/v of *phenacetin* in *chloroform*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (5 µm),
- mobile phase: a mixture of 58 volumes of *n-hexane*, 40 volumes of *chloroform*, 2 volumes of *methanol* and 0.1 volume of *glacial acetic acid*,
- flow rate: 1.8 ml per minute,
- spectrophotometer set at 243 nm,
- injection volume: 20 µl.

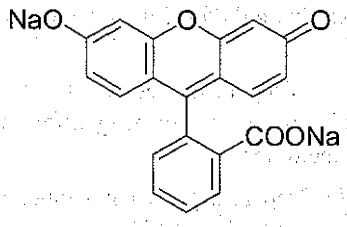
The assay is not valid unless the resolution between the peaks due to fluocinolone acetonide and phenacetin is more than 2, and the capacity factors of fluocinolone acetonide and phenacetin are about 3 and 2, respectively. If these conditions are not achieved, adjust the concentration of methanol and chloroform in the mobile phase. Repeat the adjustment of chloroform and methanol concentration until correct values for both resolution and capacity factors have been obtained.

Calculate the content of $C_{24}H_{30}F_2O_6$ in the cream.

Storage. Store at a temperature not exceeding 30°.

Fluorescein Sodium

Soluble Fluorescein



$C_{20}H_{10}Na_2O_5$ Mol. Wt. 376.3

Fluorescin Sodium is disodium 2-(3-oxo-6-oxido-3*H*-xanthen-9-yl)benzoate.

Fluorescein Sodium contains not less than 98.5 per cent and not more than 100.5 per cent of $C_{20}H_{10}Na_2O_5$, calculated on the dried basis.

Category. Diagnostic aid (dye for detection of corneal lesions and foreign bodies).

Description. An orange-red powder; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorescein sodium IPRS* or with the reference spectrum of fluorescein sodium.

B. A solution is strongly fluorescent, even in extreme dilutions. The fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

C. A drop of a 0.05 per cent w/v solution, absorbed on a piece of filter paper, colours the paper yellow. On exposing the moist paper to the vapours of bromine for 1 minute and then to the vapours of ammonia, the yellow colour becomes deep pink.

D. The residue after incineration gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 7.0 to 9.0, determined in a 2.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), using a silica gel GF254 precoated plate (such as Merck silica gel 60 GF254 plate).

Mobile phase. A mixture of 80 volumes of *chloroform* and 20 volumes of *methanol*.

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *0.1 M methanolic hydrochloric acid*.

Reference solution. A 0.002 per cent w/v solution of the substance under examination in 100.0 ml of *0.1 M methanolic hydrochloric acid*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. Expose the plate to iodine vapour for 30 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Chloroform-soluble matter. Dissolve 0.2 g in 10 ml of *0.1 M sodium hydroxide* and extract with 10 ml of *chloroform*. Dry the chloroform layer with *anhydrous sodium sulphate* and filter. Absorbance of the resulting solution at about 480 nm, using *chloroform* as the blank, not more than 0.10 (2.4.7).

Zinc. Dissolve 0.1 g in 10 ml of *water*, add 2 ml of *hydrochloric acid*, filter and add 0.1 ml of *potassium ferrocyanide solution*; no turbidity or precipitate is produced immediately.

Chlorides (2.3.12). Dissolve 75 mg in 20 ml of *water*, add 2 ml of *nitric acid* and filter; the filtrate complies with the limit test for chlorides (0.33 per cent).

Sulphates (2.3.17). Dissolve 62.5 mg in 100 ml of *water*. To 20 ml add 2.5 ml of *dilute hydrochloric acid* and filter; the filtrate complies with the limit test for sulphates (1.2 per cent).

Dimethylformamide. Determine by gas chromatography (2.4.13).

Test solution (a). Dissolve 1.0 g of the substance under examination in 10 ml of *water*, add with stirring, 10 ml of *0.6 M hydrochloric acid*, allow to stand for 15 minutes and centrifuge. To 5 ml of the supernatant liquid add 0.1 g of *trisodium phosphate* and shake to dissolve.

Test solution (b). Prepare in the same manner as test solution (a) but using 10 ml of a 0.02 per cent w/v solution of *dimethylacetamide* (internal standard) in place of *water*.

Reference solution. Mix 10 ml of a 0.02 per cent w/v solution of *dimethylformamide IPRS* with 10 ml of the internal standard.

Chromatographic system
- a glass column 1.5m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 10 per cent w/w of *polyethylene glycol 1000*,
- temperature:
  column 120°,
  inlet port and detector. 180°,
- flame ionisation detector,
- flow rate: 30 ml per minute of the carrier gas (nitrogen).

In the chromatogram obtained with test solution (b) the ratio of the area of any peak corresponding to dimethylformamide to the area of the peak due to the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution.

Resorcinol. Determine by thin-layer chromatography (2.4.17), using a silica gel GF254 precoated plate (such as Merck silica gel 60 GF254 plate).

Mobile phase. A mixture of 60 volumes of *hexane* and 40 volumes of *ethyl acetate*.

Test solution. Dissolve 1 g of the substance under examination in 10 ml of *water*, add slowly with constant stirring, 10 ml of *0.6 M hydrochloric acid*, allow to stand for 15 minutes, centrifuge and use the supernatant liquid.

Reference solution. A 0.025 per cent w/v solution of *resorcinol* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and expose to iodine vapour for 30 minutes. Any spot corresponding to *resorcinol* in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Acriflavin. Dissolve 10 mg in 5 ml of *water*, and add a few drops of *sodium salicylate solution*; no precipitate is formed.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.5 g, dissolve in 20 ml of *water*, add 5 ml of *dilute hydrochloric acid*, and extract with four quantities, each of 20 ml, of a mixture of equal volumes of *2-methyl-1-propanol* and *chloroform*. Wash the combined extracts with 10 ml of *water*, extract the washings with 5 ml of the mixture of *2-methyl-1-propanol* and *chloroform* and add to the combined extracts. Evaporate the combined extracts to dryness on a water-bath in a current of air, dissolve the residue in 10 ml of *ethanol (95 per cent)*, evaporate to dryness on a water-bath and dry to constant weight at 105°.

1 g of the residue is equivalent to 1.132 g of $C_{20}H_{10}Na_2O_5$.

Storage. Store protected from light.

Fluorescein Eye Drops

Fluorescein Sodium Eye Drops

Fluorescein Eye Drops are a sterile solution of Fluorescein Sodium in Purified Water.

Fluorescein Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluorescein sodium, $C_{20}H_{10}Na_2O_5$.

Usual strengths. 1 per cent w/v; 2 per cent w/v.

Identification

A. Evaporate a volume of the eye drops containing 20 mg of Fluorescein Sodium and dry at 105° for 30 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorescein sodium IPRS* or with the reference spectrum of fluorescein sodium.

B. Dilute the eye drops with *water* to produce a solution containing 0.05 per cent w/v of Fluorescein Sodium. One drop of the solution, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to the vapours of bromine for 1 minute and then to vapours of ammonia, the yellow colour becomes deep pink.

C. The eye drops are strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

Tests

pH (2.4.24). 7.0 to 9.0.

Related substances. Determine by thin-layer chromatography (2.4.17), using a silica gel *GF254* precoated plate (such as Merck silica gel 60 GF254 plate).

Mobile phase. A mixture of 80 volumes of *chloroform* and 20 volumes of *methanol*.

Test solution. Dilute a suitable volume of the eye drops, if necessary, with an equal volume of *0.1 M methanolic hydrochloric acid* so as it give a concentration of 1.0 per cent w/v of fluorescein sodium.

Reference solution. Dilute 1.0 ml of the test solution to 500.0 ml with *0.1 M methanolic hydrochloric acid*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. Expose the plate to iodine vapour for 30 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Chloroform-soluble matter. To a volume of the eye drops containing 0.1 g of Fluorescein Sodium add 1 ml of *2 M sodium hydroxide*, extract with 10 ml of *chloroform*, dry the chloroform layer with *anhydrous sodium sulphate* and filter; absorbance of the resulting solution at about 480 nm, using *chloroform* as the blank, not more than 0.05 (2.4.7).

Dimethylformamide. Determine by gas chromatography (2.4.13).

Test solution (a). Dilute the eye drops with *water*, if necessary, to produce a solution containing 1.0 per cent w/v of Fluorescein Sodium. To 5 ml of the solution add, with stirring, 0.3 ml of *1 M hydrochloric acid*, allow to stand for 15 minutes and centrifuge; dissolve 10 mg of *trisodium phosphate* in 2 ml of the supernatant liquid.

Test solution (b). Prepare in the same manner as test solution (a) but adding 1.0 ml of a 0.01 per cent v/v solution of *dimethylacetamide* (internal standard) before the *hydrochloric acid*.

Reference solution. A solution containing 0.002 per cent v/v of *dimethylformamide* and 0.002 per cent v/v of the internal standard.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 10 per cent w/w of *polyethylene glycol 1000*,
- temperature:
  column 120°,
  inlet port and detector. 180°,
- flame ionisation detector,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

In the chromatogram obtained with test solution (b) the ratio of the area of any peak corresponding to dimethylformamide to the area of the peak due to the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution.

Resorcinol. Determine by thin-layer chromatography (2.4.17), using a silica gel *GF254* precoated plate (such as Merck silica gel 60 GF254 plate).

Mobile phase. A mixture of 60 volumes of *hexane* and 40 volumes of *ethyl acetate*.

Test solution. Dilute the eye drops with *water*, if necessary, to produce a solution containing 1.0 per cent w/v of Fluorescein Sodium and to 10 ml add, with stirring, 2.5 ml of *0.25 M hydrochloric acid*. Allow to stand for 15 minutes, centrifuge and dissolve 0.1g of *trisodium phosphate* in 5ml of the supernatant liquid.

Reference solution. A 0.004 per cent w/v solution of *resorcinol* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and expose to iodine vapour for 30 minutes. Any spot corresponding to resorcinol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Dilute a measured volume of the eye drops containing about 0.1 g of Fluorescein Sodium to 20 ml with *water*. Add 5 ml of *dilute hydrochloric acid* and extract with four quantities, each of 20 ml, of a mixture of equal volumes of *2-methyl-1-propanol* and *chloroform*. Wash the combined extracts with 10 ml of *water*, extract the washings with 5 ml of the mixture of *2-methyl-1-propanol* and *chloroform* and add to the combined extracts. Evaporate the combined extracts to dryness on a water-bath in a current of air, dissolve the residue in 10 ml of *ethanol (95 per cent)*, evaporate to dryness on a water-bath and dry to constant weight at 105°.

1 g of the residue is equivalent to 1.132 g of $C_{20}H_{10}Na_2O_5$.

Storage. Store protected from light.

Labelling. The label states that the eye drops should be discarded after use on a single occasion.

Fluorescein Injection

Fluorescein Sodium Injection

Fluorescein Injection is a sterile solution of Fluorescein Sodium in Water for Injections.

Fluorescein Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fluorescein sodium, $C_{20}H_{10}Na_2O_5$.

Usual strengths. 100 mg per ml; 200 mg per ml.

Identification

A. Evaporate 1 ml of the injection to dryness on a water bath and dry the residue at 105° for 30 minutes. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorescein sodium IPRS* or with the reference spectrum of fluorescein sodium.

B. The injection is strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.

C. One drop of the solution containing 0.05 per cent w/v of Fluorescein Sodium, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to bromine vapour for 1 minute and then to ammonia vapour the yellow colour becomes deep pink.

Tests

pH (2.4.24). 8.0 to 9.8.

Chloroform-soluble matter. Dilute a volume of injection containing about 0.5 g of Fluorescein Sodium in 10 ml of *water*. To 4 ml of the solution add 1 ml of *1 M sodium hydroxide*, dilute to 10 ml with *water*, extract with 10 ml of *chloroform* and dry the chloroform layer over *anhydrous sodium sulphate*. The absorbance of the solution at 480 nm (2.4.7) is not more than 0.1.

Dimethylformamide. Determine by gas chromatography (2.4.13).

Test solution (a). Dilute a volume of injection containing about 0.5 g of fluorescein sodium to 10.0 ml with *water*. To this solution add, with stirring, 1 ml of *3 M hydrochloric acid*, allow to stand for 15 minutes and centrifuge; dissolve 0.1 g of *trisodium orthophosphate* in 5 ml of the supernatant liquid.

Test solution (b). Dilute a volume of injection containing 0.5 g of fluorescein sodium to 10.0 ml with *water*. To this solution add, with stirring, 1.0 ml of a 0.10 per cent v/v solution of *dimethylacetamide* (internal standard) and 1 ml of *3 M hydrochloric acid*, allow to stand for 15 minutes and centrifuge; dissolve 0.1 g of *trisodium orthophosphate* in 5 ml of the supernatant liquid.

Reference solution. A solution containing 0.002 per cent v/v each of *dimethylformamide* and *dimethylacetamide* (internal standard).

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with *acid-washed, silanised diatomaceous support* (80 to 100 mesh) coated with 10 per cent w/w of *polyethylene glycol 1000*,
- temperature :
  column 120°,
  inlet port and detector at 120°,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject the reference solution, test solution (a) and (b). In the chromatogram obtained with test solution (b) the ratio of the area of any peak corresponding to *dimethylformamide* to the area of the peak due to the internal standard is not more than the corresponding ratio in the chromatogram obtained with the reference solution (0.2 per cent).

Related substances and resorcinol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

Test solution. Dilute a volume of injection containing about 1 g of Fluorescein Sodium with *0.1 M methanolic hydrochloric acid*.

Reference solution (a). A 5.0 per cent w/v solution of *fluorescein sodium IPRS* in *water*. To 5 ml of the solution add 2 ml of *phosphate buffer pH 8.0*, 3 ml of *water* and 2.5 g of *sodium chloride*, shake to dissolve the *sodium chloride* and extract with two 25-ml quantities of *peroxide-free ether*. Dry the combined extracts over *anhydrous sodium sulphate*, evaporate to dryness under reduced pressure and dissolve the residue in 10 ml of *0.1 M methanolic hydrochloric acid*.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml *with 0.1 M methanolic hydrochloric acid*.

Reference solution (c). Dilute 2.0 ml of reference solution (b) to 5.0 ml with *0.1 M methanolic hydrochloric acid*.

Reference solution (d). A 0.0125 per cent w/v solution of *resorcinol* in *0.1 M methanolic hydrochloric acid*.

Reference solution (e). A mixture of 9 ml of reference solution (d) and 1 ml of the test solution.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose the plate to iodine vapour for 30 minutes and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (d) (0.2 per cent). On examination in daylight, any spot corresponding to resorcinol in the chromatogram obtained with reference solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (d) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (e) shows two clearly separated spots in daylight.

Other tests. Comply with the tests stated under Parenteral Preparation (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing about 1.0 per cent w/v of Fluorescein Sodium. Dilute 1.0 ml of the solution to 200.0 ml with the mobile phase.

Reference solution. Dissolve 55 mg of *diacetylfluorescein IPRS* in a mixture of 5 ml of *ethanol (95 per cent)* and 1 ml of

2.5 M sodium hydroxide, heat on a water bath for 20 minutes, mixing frequently, cool and add sufficient *water* to produce 50 ml. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 2),
- mobile phase: a mixture of 5 volumes of *triethylamine*, 400 volumes of *acetonitrile* and 595 volumes of *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution. The test is not valid unless the relative standard deviation for replicate injections is not less than 2.0 per cent.

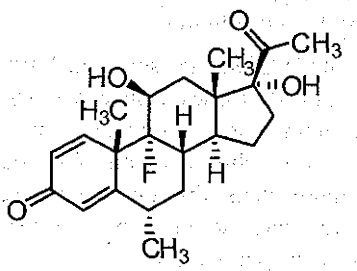
Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{10}Na_2O_5$ in the injection.

1 mg of anhydrous diacetylfluorescein is equivalent to 0.9037 mg of $C_{20}H_{10}Na_2O_5$.

Storage. Store protected from light.

Fluorometholone



$C_{22}H_{29}FO_4$ Mol. Wt. 376.5

Fluorometholone is 9α-Fluoro-11β,17α-dihydroxy-6 α-methylpregna-1,4-diene-3,20-dione

Fluorometholone contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{22}H_{29}FO_4$, calculated on the dried basis.

Category. Glucocorticoid.

Description. A white to yellowish white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *fluorometholone IPRS* or with the reference spectrum of fluorometholone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Specific optical rotation (2.3.22). +52.0° to +60.0°, determined on 1.0 per cent w/v solution in *pyridine*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in *methanol* and dilute to 100.0 ml with *methanol*.

Reference solution (a). A 0.00005 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (b). A solution containing 0.00005 per cent w/v each of *deltamedrane IPRS* and *fluorometholone IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 40 volumes of *water* and 60 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to deltamedrane and fluorometholone is not less than 1.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying at 60° at 0.7 kPa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5 mg of the substance under examination in *methanol* and dilute to 100.0 ml with *methanol*.

Reference solution (a). A 0.005 per cent w/v solution of *fluorometholone IPRS* in *methanol*.

Reference solution (b). A solution containing 0.00005 per cent w/v, each of, *deltamedrane IPRS* and *fluorometholone IPRS* in *methanol*.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to deltamedrane and fluorometholone is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{29}FO_4$.

Fluorometholone Eye Drops

Fluorometholone Eye Drops are a sterile suspension of Fluorometholone in Purified Water.

Fluorometholone Eye Drops contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fluorometholone, $C_{22}H_{29}FO_4$.

Usual strength. 0.1 per cent w/v.

Identification

A. Shake a quantity of the eye drops containing 5 mg of Fluorometholone with 20 ml of *acetone*, filter and evaporate the filtrate to dryness. Dissolve the residue in 10 ml of *acetone*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorometholone IPRS* or with the reference spectrum of fluorometholone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 6.0 to 7.5.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water*.

Test solution. Dilute a volume of the eye drops containing 1 mg of Fluorometholone to 10.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 20 ml with *methanol*. Further dilute 1.0 ml of the solution to 10.0 ml with *methanol*.

Reference solution (b). A solution containing 0.00005 per cent w/v each of *deltamedrane IPRS* and *fluorometholone IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 40 volumes of *water* and 60 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to deltamedrane and fluorometholone is not less than 1.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water*.

Test solution. Dilute a volume of eye drops containing 1 mg of Fluorometholone to 25.0 ml with the solvent mixture.

Reference solution (a). Dilute 4.0 ml of a 0.05 per cent w/v solution of *fluorometholone IPRS* in *methanol* to 50.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.00005 per cent w/v each of *deltamedrane IPRS* and *fluorometholone IPRS* in the solvent mixture.

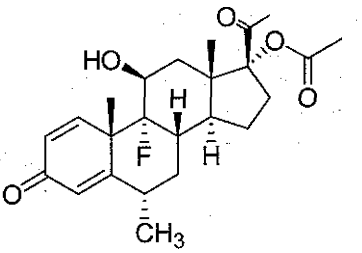
Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to deltamedrane and fluorometholone is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{29}FO_4$ in the eye drops.

Fluorometholone Acetate



$C_{24}H_{31}FO_5$ Mol. Wt. 418.5

Fluorometholone Acetate is Pregna-1,4-diene-3,20-dione, 17-(acetyloxy)-9-fluoro-11-hydroxy-6-methyl-(6α,11β); 9-fluoro-11β,17-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione, 17 acetate.

Fluorometholone Acetate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{24}H_{31}FO_5$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white to yellowish crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorometholone acetate IPRS* or with the reference spectrum of fluorometholone acetate.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows absorption maxima as obtained with *fluorometholone acetate IPRS* of the same concentration.

Tests

Specific optical rotation (2.4.22). +25.0° to +31.0°, determined in a 2.0 per cent w/v solution in *chloroform*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of *acetonitrile*.

Reference solution (a). A 0.003 per cent w/v solution of *fluorometholone IPRS* in minimum quantity of *methanol* and dilute to volume with *acetonitrile*.

Reference solution (b). A 0.1 per cent w/v solution of *fluorometholone acetate IPRS* in *acetonitrile*.

Reference solution (c). Mix a suitable quantity of reference solution (a) and reference solution (b) to obtain a 0.003 per cent w/v solution each of *fluorometholone IPRS* and *fluorometholone acetate IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of a *water* and 40 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Fluorometholone	0.6	1.0
Compound 5OX[1]	0.89	1.0
Fluorometholone Acetate	1.0	—
Fluorometholone Diacetate	1.39	2.2
Fluorometholone Acetate, epoxy analog[2]	1.58	1.0
Fluorometholone Acetate, Delta 9(11)[3]	1.82	1.0
Fluorometholone Acetate, 7,9(11) Diene[4]	1.77	0.55

[1]19b,11b-Epoxy-17-hydroxy-6a-methylpregna-1,4-dien-3,20-dione,

[2]17-Acetoxy-9b,11b-epoxy-6a-methylpregna-1,4-dien-3,20-dione,

[3]17a-Acetoxy-6a-methylpregna-1,4,9(11)-trien-3,20-dione,

[4]17a-Acetoxy-6a-methylpregna-1,4,7,9(11)-tetraen-3,20-dione,

a relative to fluorometholone.

Inject reference solution (b) and (c). The test is not valid unless the resolution between fluorometholone acetate and fluorometholone peaks is not less than 10.0 in the chromatogram obtained with reference solution (c), the column efficiency is not less than 10000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (b).

Inject the test solution. Run the chromatogram at least 2.5 times of the retention time of the principal peak. The area of any peak corresponding to fluorometholone and fluorometholone diacetate is not more than 1.0 per cent, the area of any peak corresponding to compound 5OX, fluorometholone acetate and epoxy analog is not more than 0.5 per cent, fluorometholone acetate, delta 9(11) is not more than 0.2 per cent, fluorometholone acetate, 7,9(11) diene is not more than 0.3 per cent. The area of any other secondary peak is not more than 0.1 per cent, and the sum of areas of all the secondary peaks is not more than 1.5 per cent calculated by area normalization.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of *acetonitrile*.

Reference solution (a). A 0.1 per cent w/v solution of *fluorometholone acetate IPRS* in *acetonitrile*.

Reference solution (b). A 0.1 per cent w/v solution of *fluorometholone IPRS* in minimum quantity of *methanol* and dilute in *acetonitrile* and mix equal volume of the solution and the reference solution (a), dilute with *acetonitrile* to a final concentration of about 0.003 per cent w/v solution each for *fluorometholone IPRS* and *fluorometholone acetate IPRS*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of a *water* and 40 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between fluorometholone acetate and fluorometholone peaks is not less than 10.0 with reference solution (b), the column efficiency is not less than 10000

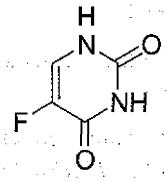
theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{24}H_{31}FO_5$.

Storage. Store protected from moisture.

Fluorouracil



$C_4H_3FN_2O_2$ Mol. Wt. 130.1

Fluorouracil is 5-fluoro-1*H*,3*H*-pyrimidine-2,4-dione.

Fluorouracil contains not less than 98.0 per cent and not more than 101.0 per cent of $C_4H_3FN_2O_2$, calculated on the dried basis.

Category. Anticancer.

Description. A white or almost white, crystalline powder.

CAUTION - Great care should be taken to avoid inhaling particles of Fluorouracil and exposing the skin to it.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorouracil IPRS* or with the reference spectrum of fluorouracil.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *acetate buffer pH 4.7* shows an absorption maximum only at about 266 nm.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. To 5 ml of a 1 per cent w/v solution add 1 ml of *bromine water*; the colour of bromine is discharged.

Tests

Appearance of solution. A 1.0 per cent w/v solution in carbon *dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 or BYS7 (2.4.1).

pH (2.4.24). 4.5 to 5.0, determined in a 1.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 70 volumes of *ethyl acetate*, 15 volumes of *methanol* and 15 volumes of *water*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml of *methanol (50 per cent)*.

Test solution (b). Dilute 5.0 ml of the test solution to 25.0 ml with *methanol (50 per cent)*.

Reference solution (a). A 0.2 per cent w/v solution of *fluorouracil IPRS* in *methanol (50 per cent)*.

Reference solution (b). A 0.0025 per cent w/v solution of *fluorouracil IPRS* in *methanol (50 per cent)*.

Reference solution (c). A 0.0025 per cent w/v solution of *5-hydroxyuracil* in *methanol (50 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a freshly prepared 0.5 per cent w/v solution of *fast blue B salt* and then with *0.1 M sodium hydroxide*. Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c). Ignore any secondary spot on or near the line of application.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 1.0 g in a platinum crucible.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* in an oven at 80° at a pressure of 1.5 to 2.5 kPa for 4 hours.

Assay. Weigh 0.2 g, dissolve in 80 ml of *dimethylformamide* with the aid of gentle heat and cool. Titrate with *0.1 M tetrabutylammonium hydroxide* in *methanol*, using 0.25 ml of a 1 per cent w/v solution of *thymol blue* in *dimethylformamide* as indicator. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01301 g of $C_4H_3FN_2O_2$.

Storage. Store protected from light.

Fluorouracil Injection

Fluorouracil Injection is a sterile solution in Water for Injections of fluorouracil sodium, prepared by the interaction of Fluorouracil and Sodium Hydroxide.

Fluorouracil Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluorouracil, $C_4H_3FN_2O_2$.

Usual strengths. 25 mg per ml; 50 mg per ml.

Description. A colourless or almost colourless solution.

Identification

A. Acidify carefully a volume of the injection containing 0.1 g of Fluorouracil with *glacial acetic acid*, stir, cool and filter. Wash the precipitate with 1 ml of *water* and dry over *phosphorus pentoxide* at 80° at a pressure of 2 kPa for 4 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluorouracil IPRS* or with the reference spectrum of fluorouracil.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 266 nm.

C. To a volume of the injection containing 50 mg of Fluorouracil add 1 ml of *bromine water*; the colour of bromine is discharged.

Tests

pH (2.4.24). 8.5 to 9.5.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 70 volumes of *ethyl acetate*, 15 volumes of *methanol* and 15 volumes of *water*.

Test solution. Dilute a suitable quantity of the injection with *water* to produce a solution containing the equivalent of 2 per cent w/v of Fluorouracil.

Reference solution (a). Dilute 1 volume of test solution to 400 volumes with *methanol (50 per cent)*.

Reference solution (b). A 0.005 per cent w/v solution of *5-hydroxyuracil* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray with a freshly prepared solution containing 0.5 per cent w/v of *fast blue B salt* and then with *0.1 M sodium hydroxide*. Any spot corresponding to 5-hydroxyuracil in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any secondary spot on or near the line of application.

Urea. Carry out the method described under Related substances applying separately to the plate 20 µl of the following solutions. For the test solution dilute a suitable quantity of the injection with *water* to produce a solution containing the equivalent of 0.5 per cent w/v of Fluorouracil. The reference solution contains 0.02 per cent w/v of *urea* in *water*. After development, dry the plate in air, spray with a mixture of 10 volumes of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *ethanol (95 per cent)* and 1 volume of *hydrochloric acid* and heat at 105° until maximum intensity of the spots is obtained. Any spot corresponding to urea in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Bacterial endotoxins (2.2.3). Not more than 0.33 Endotoxin Unit per mg of fluorouracil.

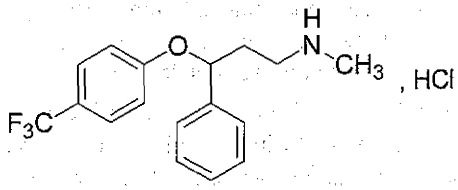
Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume containing about 50 mg of Fluorouracil add 20 ml of *1 M hydrochloric acid* and sufficient *water* to produce 250.0 ml. Dilute 5.0 ml to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 266 nm (2.4.7). Calculate the content of $C_4H_3FN_2O_2$ taking 552 as the specific absorbance at 266 nm.

Storage. Store protected from light in single dose containers at a temperature not exceeding 30°. The injection should not be allowed to freeze.

Labelling. The label states (1) the strength in terms of the equivalent amount of Fluorouracil in a suitable dose-volume; (2) that, if separation has occurred, the injection should be heated to 60°, shaken vigorously and allowed to cool to body temperature prior to use.

Fluoxetine Hydrochloride



$C_{17}H_{18}F_3NO,HCl$ Mol. Wt. 345.8

Fluoxetine Hydrochloride is (*RS*)-*N*-methyl-3-phenyl-3-[4-trifluoromethyl)phenoxy]propylamine hydrochloride.

Fluoxetine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{18}F_3NO,HCl$, calculated on the anhydrous basis.

Category. Antidepressant.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluoxetine hydrochloride IPRS* or with the reference spectrum fluoxetine hydrochloride.

B. It gives the reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. Dissolve 2.0 g in a mixture of 15 volumes of *water* and 85 volumes of *methanol* and dilute to 100 ml with the same solvent mixture (solution A). Solution A is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 4.5 to 6.5. determined in a solution of 0.2 g in sufficient *carbon dioxide-free water* to produce 20 ml.

Optical rotation (2.4.22). –0.05° to +0.05°, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 0.055 g of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Test solution (b). Dilute 2.0 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution. Dissolve 0.022 g of *fluoxetine hydrochloride IPRS* in 10.0 ml of *0.5 M sulphuric acid*. Heat at about 85° for 3 hours. Allow to cool. The resulting solution contains mainly of (1*RS*)-3-(methylamino)-1-phenylpropan-1-ol (fluoxetine impurity A) and some 4-trifluoromethylphenol. To 0.4 ml of the solution add 28 mg of *fluoxetine hydrochloride IPRS*, about 1 mg of *N-methyl-3-phenylpropan-1-amine IPRS* (*fluoxetine impurity B IPRS*) and about 1 mg of *(3RS)-N-methyl-3-phenyl-3-[3-(trifluoromethyl) phenoxy]propan-1-amine IPRS* (*fluoxetine impurity C IPRS*) and dilute to 25.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 8 volumes of *methanol*, 30 volumes of *tetrahydrofuran* and 62 volumes of a solution of *triethylamine* prepared by adding 980 ml of *water* to 10 ml of *triethylamine*, mixing and adjusted to pH 6.0 with *orthophosphoric acid* and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 10 µl.

Inject the reference solution. The relative retention time with respect to fluoxetine for fluoxetine impurity A is about 0.24, for fluoxetine impurity B is about 0.27 and for fluoxetine impurity C is about 0.94.

The test is not valid unless the retention time of the peak due to fluoxetine is 10-18 minutes; the retention time of the peak due to 4-trifluoromethylphenol is not greater than 35 minutes (if no peak due to 4-trifluoromethylphenol is seen in the chromatogram, inject a 0.02 per cent solution of *4-trifluoromethylphenol* in the mobile phase); the peak to valley ratio for fluoxetine impurity C is not less than 11. If the ratio is less than 11, reduce the volume of methanol and increase the volume of the solution of triethylamine in the mobile phase.

Inject test solution (a) and (b). Continue the chromatography for 3 times the retention time of fluoxetine. In the chromatogram obtained with test solution (b), the area of any peak due to fluoxetine impurity C is not greater than 0.0015 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.15 per cent).

In the chromatogram obtained with test solution (a) the areas of any peaks due to fluoxetine impurity A and fluoxetine impurity B are not greater than 0.0125 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.25 per cent); none of the peaks other than the principal peak and the peaks due to fluoxetine impurity A and fluoxetine impurity B, has an area greater than 0.005 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.1 per cent);the sum of the areas of all the peaks, other than the principal peak, is not greater than 0.025 times the area of the principal peak obtained with test solution (b) (0.5 per cent). Ignore any peak with an area less than 0.0025 times that of the principal peak in the chromatogram obtained with test solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

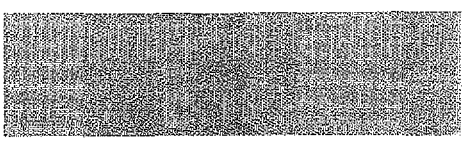
Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 0.055 g of the substance under examination, dissolve in the mobile phase and add sufficient mobile phase to produce 50.0 ml. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. Weigh 0.055 g of *fluoxetine hydrochloride IPRS* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Use the chromatographic system described under test for Related substances and set the spectrophotometer at 227 nm.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is at least 50 per cent of the full scale of the recorder.



Adjust the volumes of methanol and the solution of triethylamine in the mobile phase so that the retention time of fluoxetine is between 10 minutes and 18 minutes.

The assay is not valid unless the symmetry factor calculated at 10 per cent of the height of the peak due to fluoxetine is at most 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{18}F_3NO,HCl$.

Fluoxetine Capsules

Fluoxetine Hydrochloride Capsules

Fluoxetine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluoxetine, $C_{17}H_{18}F_3NO$.

Usual strengths. 10 mg; 20 mg.

Identification

A. Shake a quantity of the contents of the capsules containing 10 mg of fluoxetine with 10 ml of *methanol*, centrifuge for 10 minutes and filter. Evaporate the filtrate to dryness with the aid of a current of air and mild heat. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluoxetine hydrochloride IPRS* or with the reference spectrum of fluoxetine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Diethylamine phosphate suspension. To 250 ml of *acetonitrile*, add 1.0 ml of *diethylamine*, mix, and adjusted to pH 3.5 with *orthophosphoric acid*.

NOTE — Keep the suspension well-mixed.

Test solution. To 5.0 ml of the filtrate obtained as given above, add 2.0 ml of the diethylamine phosphate suspension and mix well.

Reference solution. Dissolve 0.022 g of *fluoxetine hydrochloride IPRS* in sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and mix. Dilute 10.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid*. To 5.0 ml of the resulting solution, add 2.0 ml of the diethylamine phosphate suspension and mix well.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with particles of silica the surface of which has been modified with chemically-bonded cyano groups (5 µm),
- mobile phase: a mixture of 0.4 volume of *diethylamine*, 40 volumes of *acetonitrile* and 60 volumes of *water*, adjusted to pH 3.5 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Q. Not less than 70 per cent of the stated amount of $C_{17}H_{18}F_3NO$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the contents of the capsules containing 20 mg of fluoxetine, disperse in 10.0 ml of the mobile phase, mix and centrifuge.

Reference solution. A solution of *fluoxetine hydrochloride IPRS* equivalent to 0.001 per cent w/v of fluoxetine in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with particles of silica the surface of which has been modified with chemically-bonded cyano groups (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a solution of *triethylamine*, prepared by adding 10 ml of *triethylamine* to 980 ml of *water*, mixing, adjusted to pH 6.0 with *orthophosphoric acid* (about 4.5 ml) and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1100 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (0.5 per cent)

and the sum of areas of all secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peaks with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the contents of the capsules containing about 20 mg of fluoxetine, disperse in 200.0 ml of the mobile phase, mix and filter.

Reference solution. A 0.011 per cent w/v solution of *fluoxetine hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 7.5 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 33 volumes of a solution containing 0.3 per cent w/v of *glacial acetic acid* and 0.64 per cent w/v of *sodium pentanesulphonate*, adjusted to pH 5.0 with *5 M sodium hydroxide*, and 67 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 227 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{18}F_3NO$ in the capsules.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of fluoxetine.

Fluoxetine Oral Solution

Fluoxetine Oral Liquid; Fluoxetine Hydrochloride Oral Solution

Fluoxetine Oral Solution is a solution of Fluoxetine Hydrochloride in a suitable aqueous vehicle. It may contain one or more preservatives.

Fluoxetine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluoxetine, $C_{17}H_{18}F_3NO$.

Usual strength. 20 mg per 5 ml.

Identification

Transfer a volume of the oral solution containing about 20 mg of fluoxetine, to a separating funnel, add 5 ml of *water* and 0.5 ml of *1 M sodium hydroxide;* extract with 5 ml of *chloroform* and discard the aqueous layer. Evaporate the chloroform layer to dryness. The residue dissolved in 0.4 ml of *chloroform* complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluoxetine hydrochloride IPRS* treated in the same manner.

Tests

pH (2.4.24). 2.5 to 4.5.

Related substances. Determine by liquid chromatography (2.4.14).

Ion pair solution. Dissolve about 4.3 g of *sodium 1-octanesulphonate* and 13.8 g of *monobasic sodium phosphate* in 1000 ml of *water*, and adjusted to pH 3.0 with *orthophosphoric acid*.

Solvent mixture. 60 volumes of *ion pair solution*, 30 volumes of *methanol* and 10 volumes of *acetonitrile*.

Test solution (a) Weigh a quantity of the oral solution containing 19 mg of fluoxetine and dilute to 10.0 ml with the solvent mixture.

Test solution (b). Dilute 1.0 ml of test solution (a) to 25.0 ml with the solvent mixture.

Reference solution. A 0.2 per cent w/v solution of *fluoxetine hydrochloride IPRS* in *1 M sulphuric acid*. Heat this solution at 85° for 1 hour. To 1.0 ml of the solution add about 10 mg *fluoxetine hydrochloride IPRS*, dissolve in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase : A. a mixture of 53 volumes of *ion-pair solution*, 26 volumes of *methanol* and 21 volumes of *acetonitrile*,

  B. a mixture of 43 volumes of *ion-pair solution*, 35 volumes of *acetonitrile* and 22 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
13	100	0
15	0	100
29	0	100
30	100	0

Inject the reference solution. The retention time of any secondary peak is not more than 13 minutes.

Inject test solution (a) and (b). In the chromatogram obtained with test solution (a), the area of any secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.4 per cent) and the sum of the areas of the peaks, other than the principal peak, is not more than 0.2 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.8 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the oral solution containing 4.0 mg of fluoxetine and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.0045 per cent w/v solution of *fluoxetine hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (10 µm),
- mobile phase: a mixture of equal volumes of a buffer solution prepared by adding 10 ml of *triethylamine* to 980 ml with *water* and adjusted to pH 6.0, and *acetonitrile,*
- flow rate 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject the reference solution. The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_{17}H_{18}F_3NO$, weight in volume.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of fluoxetine.

Fluoxetine Tablets

Fluoxetine Hydrochloride Tablets

Fluoxetine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluoxetine, $C_{17}H_{18}F_3NO$.

Usual strengths. 10 mg; 20 mg.

Identification

A. Shake a quantity of the contents of the tablets containing 10 mg of fluoxetine with 10 ml of *methanol*, centrifuge for 10 minutes and filter. Evaporate the filtrate to dryness with the aid of a current of air and mild heat. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluoxetine hydrochloride IPRS* or with the reference spectrum of fluoxetine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Diethylamine phosphate suspension. To 250 ml of *acetonitrile* add 1.0 ml of *diethylamine*, mix, and adjusted to pH 3.5 with *orthophosphoric acid.*

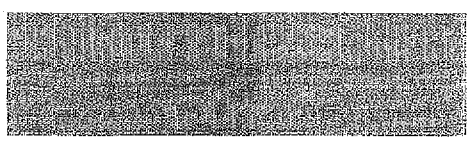
NOTE—Keep the suspension well-mixed.

Test solution. To 5.0 ml of the filtrate obtained as given above, add 2.0 ml of the diethylamine phosphate suspension and mix well.

Reference solution. Dissolve 0.022 g of *fluoxetine hydrochloride IPRS* in sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and mix. Dilute 10.0 ml of the solution to 100.0 ml with *0.1 M hydrochloric acid.* To 5.0 ml of the resulting solution, add 2.0 ml of the diethylamine phosphate suspension and mix well.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with particles of silica the surface of which has been modified with chemically-bonded cyano groups (5 µm),
- mobile phase: a mixture of 0.4 volume of *diethylamine*, 40 volumes of *acetonitrile* and 60 volumes of *water*, adjusted to pH 3.5 with *orthophosphoric acid,*
- flow rate: 2 ml per minute,
- spectrophotometer set at 226 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



Inject the reference solution and the test solution.

Q. Not less than 70 per cent of the stated amount of $C_{17}H_{18}F_3NO$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of fluoxetine, disperse in 10.0 ml of the mobile phase, mix and centrifuge.

Reference solution. A solution of *fluoxetine hydrochloride IPRS* containing the equivalent of 0.001 per cent w/v of fluoxetine in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with particles of silica the surface of which has been modified with chemically-bonded cyano groups (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a solution of *triethylamine*, prepared by adding to 10 ml of *triethylamine* 980 ml of *water*, mixing, adjusted to pH 6.0 with *orthophosphoric acid* (about 4.5 ml) and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1100 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peaks with an area 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of fluoxetine, disperse in 200.0 ml of the mobile phase, mix and filter.

Reference solution. A 0.011 per cent w/v solution of *fluoxetine hydrochloride IPRS* in the mobile phase

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 33 volumes of a solution containing 0.3 per cent w/v of *glacial acetic acid* and 0.64 per cent w/v of *sodium pentanesulphonate*, adjusted to pH 5.0 with *5 M sodium hydroxide*, and 67 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 227 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

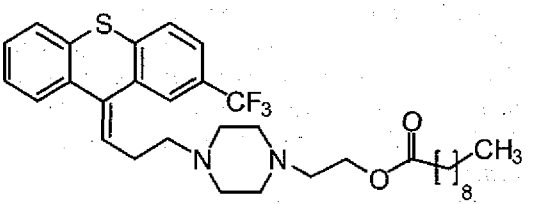
Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{18}F_3NO$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of fluoxetine.

Flupentixol Decanoate



$C_{33}H_{43}F_3N_2O_2S$ Mol. Wt. 588.8

Flupentixol Decanoate is (*Z*)-2-{4-[3-(2-Trifluoromethyl thioxanthen-9-ylidene)propyl]piperazin-1-yl}ethyl decanoate

Flupentixol Decanoate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{33}H_{43}F_3N_2O_2S$, calculated on the dried basis.

Category. Antipsychotic (Neuroleptic).

Description. A yellow, viscous oil.

Identification

A. When examined in the range 210 nm to 350 nm (2.4.7), a 0.0015 per cent w/v solution in *ethanol (95 per cent)* exhibits two maxima at 230 nm and 264 nm and the absorbances at the maxima are about 0.85 and about 0.35 respectively.

B. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *flupentixol decanoate IPRS* or with the reference spectrum of flupentixol decanoate.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Dissolve 0.25 g of the substance under examination in 100.0 ml of *acetonitrile*.

Reference solution (a). A 0.000625 per cent w/v solution of cis-*flupentixol IPRS* in *acetonitrile*.

Reference solution (b). A 0.000625 per cent w/v solution of *2-trifluoromethylthioxanthone IPRS* in *acetonitrile*.

Reference solution (c). A 0.0025 per cent w/v solution of trans-*flupentixol decanoate dihydrochloride IPRS* in *acetonitrile*.

Reference solution (d). A solution containing 0.25 per cent w/v of the substance under examination and 0.000625 per cent w/v each of cis-*flupentixol IPRS* and *2-trifluoromethyl-thioxanthone IPRS* and 0.0025 per cent w/v of *trans-flupentixol decanoate dihydrochloride IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 0.1 volume of *orthophosphoric acid*, 25 volumes of a 20 mM solution of *dioctyl sodium sulphosuccinate* prepared by dissolving 8.89 g of *dioctyl sodium sulphosuccinate* in 500 ml of *water*, stirring for 6 to 8 hours and diluting to 1000 ml with *water* and 75 volumes of *ethanol (95 per cent)*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

The substances are eluted in the following order: 2-trifluoromethylthioxanthone, *cis*-flupentixol (free alcohol), flupentixol decanoate and *trans*-flupentixol decanoate.

Inject reference solution (d). The test is not valid unless the peaks due to 2-trifluoromethylthioxanthone, *cis*-flupentixol, flupentixol decanoate and *trans*-flupentixol decanoate are clearly separated.

Inject reference solution (a), (b), (c) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to *cis*-flupentixol is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent). The area of any peak corresponding to 2-trifluoromethyl-thioxanthone is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent). The area of any peak corresponding to *trans*-flupentixol decanoate dihydrochloride is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on1.0 g by drying at 60° at a pressure of 0.7 kPa for 3 hours.

Assay. Dissolve 0.2 g in 50 ml of *anhydrous acetic acid* and determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02944 g of $C_{33}H_{43}F_3N_2O_2S$.

Storage. Store protected from light and at a temperature below –15°.

Flupentixol Injection

Flupentixol Decanoate Injection

Flupentixol Injection is a sterile solution of Flupentixol Decanoate in a suitable vegetable oil.

Flupentexol Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of flupentixol decanoate, $C_{33}H_{43}F_3N_2O_2S$.

Usual strength. 20 mg per ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 3 volumes of *diethylamine* and 90 volumes of *cyclohexane*.

Test solution. Dilute the injection to obtain a solution containing 0.4 per cent w/v of Flupentixol Decanoate with *ethanol (95 per cent)*.

Reference solution. A 0.4 per cent w/v solution of *flupentixol decanoate dihydrochloride IPRS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. After removal of the plate, allow it to dry in air, spray with a 1.0 per cent w/v solution of *sodium molybdate* in *sulphuric acid*, heat at 110° for 20 minutes and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Store protected from light.

Test solution. Dilute the injection to obtain a solution containing 0.2 per cent w/v of Flupentixol Decanoate in *acetonitrile*.

Reference solution (a). A 0.006 per cent w/v solution of *cis-flupentixol IPRS* in *acetonitrile.*

Reference solution (b). A 0.001 per cent w/v solution of *2- trifluoromethylthioxanthone IPRS* in *acetonitrile.*

Reference solution (c). A 0.002 per cent w/v solution of *trans-flupentixol decanoate IPRS* in *acetonitrile.*

Reference solution (d). A solution containing 0.006 per cent w/v of *cis-flupentixol IPRS* and 0.001 per cent w/v each of *2-trifluoromethylthioxanthone IPRS* and *trans-flupentixol decanoate IPRS* in the test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 0.1 volume of *orthophosphoric acid*, 25 volumes of a 20 mM solution of *dioctyl sodium sulphosuccinate* prepared by dissolving 8.89 g of *dioctyl sodium sulphosuccinate* in 500 ml of *water*, stirring for 6 to 8 hours and diluting to 1000 ml with *water* and 75 volumes of *ethanol (95 per cent)*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

The substances are eluted in the following order: 2-trifluoromethylthioxanthone, *cis*-flupentixol (free alcohol), flupentixol decanoate and *trans*-flupentixol decanoate.

Inject reference solution (d). The test is not valid unless the chromatogram shows clearly separated peaks.

Inject reference solution (a), (b), (c) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to *cis*-flupentixol is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent), the area of any peak corresponding to 2- trifluoro-methylthioxanthone is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the area of any peak corresponding to *trans*-flupentixol decanoate is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

Other tests. Comply with the tests stated under Parenteral preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Dilute a volume of the injection to obtain a solution containing 0.2 per cent w/v of Flupentixol Decanoate in *acetonitrile.*

Reference solution. A 0.22 per cent w/v solution of *flupentixol decanoate dihydrochloride IPRS* in *acetonitrile.*

Use chromatographic system as described under Related substances.

Inject the reference solution and the test solution.

Calculate the content of $C_{33}H_{43}F_3N_2O_2S$ in the injection.

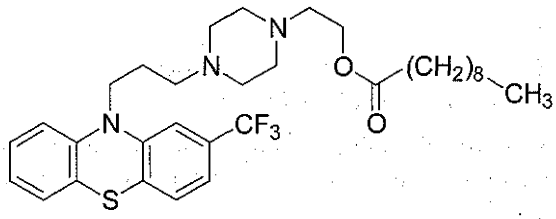
1 mg of flupentixol decanoate dihydrochloride is equivalent to 0.8897 mg of flupentixol decanoate.

Storage. Store protected from light.

Labelling. The label states that the injection is for intramuscular use.

Fluphenazine Decanoate

Fluphenazine Decanoate Ester



$C_{32}H_{44}F_3N_3O_2S$ Mol. Wt. 591.8

Fluphenazine Decanoate is 2-{4-[3-(2-trifluoro-methylphenothiazin-10-yl)propyl]piperazin-1-yl}ethyl decanoate.

Fluphenazine Decanoate contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{32}H_{44}F_3N_3O_2S$, calculated on the dried basis.

Category. Antipsychotic.

Description. A pale yellow, viscous liquid or yellow, crystalline, oily solid.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluphenazine decanoate IPRS* or with the reference spectrum of fluphenazine decanoate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol* shows an absorption maximum at about 261 nm and a less well-defined maximum at about 310 nm; absorbance at about 261 nm, about 0.60.

C. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*. Impregnate the dry plate by placing it in a tank containing a 5 per cent

v/v solution of *n-tetradecane* in *n-hexane*, allowing the impregnating solvent to ascend to the top and allowing to dry.

Mobile phase. Methanol (90 per cent).

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent)*.

Reference solution. A 2.0 per cent w/v solution of *fluphenazine decanoate IPRS* in *ethanol (95 per cent)*.

Apply to the plate 1 µl of each solution. After development, dry the plate dry in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve 5 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes; a reddish-brown colour is produced.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Carry out the test protected from light and prepare the solutions immediately before use.

Test solution. Dissolve 10 mg of the substance under examination in *acetonitrile* and dilute to 50.0 ml with *acetonitrile*.

Reference solution (a). A solution containing 0.01 per cent w/v each of *fluphenazine impurity D IPRS* and *fluphenazine impurity C IPRS* in *acetonitrile*.

Reference solution (b). Dilute 5.0 ml of the test solution to 100.0 ml with a mixture of 5 volumes of mobile phase A and 95 volumes of mobile phase B. Dilute 1.0 ml of the solution to 10.0 ml with the same solvent mixture.

Reference solution (c). A solution containing 0.00024 per cent w/v of *fluphenazine dihydrochloride IPRS* and 0.0001 per cent w/v of *fluphenazine sulphoxide IPRS* in a mixture of 5 volumes of *water* and 95 volumes of *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 1.0 per cent w/v solution of *ammonium carbonate*, adjusted to pH 7.5 with *dilute hydrochloric acid*,
  B. a mixture of 7.5 volumes of mobile phase A, 45 volumes of *acetonitrile* and 45 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	20	80
7	20	80
17	0	100
80	0	100
82	20	80

Name	Relative retention time
Fluphenazine impurity A[1]	0.13
Fluphenazine impurity B[2]	0.33
Fluphenazine impurity C[3]	0.76
Fluphenazine impurity D[4]	0.82
Fluphenazine decanoate (Retention time: about 34 minutes)	1.0

[1]fluphenazine S-oxide,

[2]fluphenazine,

[3]fluphenazine enantate,

[4]fluphenazine octanoate.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to fluphenazine impurity C and fluphenazine impurity D is not less than 6.0.

Inject reference solution (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to fluphenazine impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent) and the area of peak corresponding to fluphenazine impurity B is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Weigh 0.6 g and dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02959 g of $C_{32}H_{44}F_3N_3O_2S$.

Storage. Store protected from light.

Fluphenazine Decanoate Injection

Fluphenazine Decanoate Injection is a sterile solution of Fluphenazine Decanoate in Sesame Oil.

Fluphenazine Decanoate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluphenazine decanoate, $C_{32}H_{44}F_3N_3O_2S$.

Usual strengths. 25 mg per ml; 100 mg per ml.

Identification

A. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. *Chloroform* for the first development and *methanol (90 per cent)* for the second development.

Test solution. Dilute a suitable volume of the injection with *ethanol (95 per cent)* to produce a solution containing 2.5 mg of Flufenazine Decanoate per ml.

Reference solution. A 0.25 per cent w/v solution of *fluphenazine decanoate IPRS* in *ethanol (95 per cent)*.

Apply to the bottom right-hand corner of the plate 10 µl of the test solution. After development, dry the plate in air, turn the plate through 90° in a clockwise direction, impregnate the coating with a 5 per cent v/v solution of *n-tetradecane* in *n-hexane* and allow it to dry in air. Apply to the bottom right-hand corner of the plate, to the right of the solvent front of the first development, 10 µl of the reference solution. After the second development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Shake a volume of the injection containing 5 mg of Fluphenazine Decanoate with 1 ml of a 1 per cent w/v solution of *sucrose* in *hydrochloric acid* and allow to stand for 5 minutes; a red colour is produced in the acid layer.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Protect the solutions from light.

Test solution. Dilute a volume of the injection containing about 0.5 g Fluphenazine Decanoate in sufficient *chloroform* and dilute to 100.0 ml with *chloroform*. Dilute 1.0 ml of the solution to 25.0 ml with *acetonitrile*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*.

Reference solution (b). Add 0.05 ml of *10 M sodium hydroxide* to 2 ml of the test solution and allow to stand for 48 hours before use (generates fluphenazine impurity).

Reference solution (c). Add 0.05 ml of *hydrogen peroxide solution (200 volumes)* to 5 mg of *fluphenazine decanoate IPRS*, allow to stand for 10 minutes, add sufficient *chloroform* to produce 1 ml, mix and dilute to 100 ml with *acetonitrile* (generates mono- and di-*N*-oxide impurities).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: add 450 ml of *acetonitrile* to a mixture of 75 volumes of a 1.0 per cent w/v solution of *ammonium carbonate* and 450 volumes of *methanol*, adjusted to pH 7.5 with *1 M acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to mono-*N*- oxide and di-*N*-oxide is not less than 2.0.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to fluphenazine impurity in the chromatogram obtained with reference solution (b) is not more than 4 times the area of the peak in the chromatogram obtained with reference solution (a) (4.0 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of areas of all the secondary peaks excluding the peak of fluphenazine impurity is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with a relative retention time with reference to fluphenazine decanoate of 0.2 or less.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *Carry out the following procedure protected from light.*

Measure a volume of the injection containing 0.25 g of Fluphenazine Decanoate and dilute with 75 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

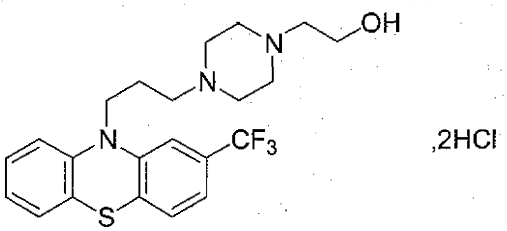
1 ml of *0.1 M perchloric acid* is equivalent to 0.02959 g of $C_{32}H_{44}F_3N_3O_2S$.

Storage. Store protected from light.

Labelling. The label states that the injection is for intramuscular injection only.

Fluphenazine Hydrochloride

Fluphenazine Dihydrochloride



$C_{22}H_{26}F_3N_3OS,2HCl$ Mol. Wt. 510.5

Fluphenazine Hydrochloride is 2-{4-[3-(2-trifluoromethylphenothiazin-10-yl)propyl]piperazin-1-yl}ethanol dihydrochloride.

Fluphenazine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent w/v of $C_{22}H_{26}F_3N_3OS,2HCl$, calculated on the dried basis.

Category. Antipsychotic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Dissolve 0.1 g in 10 ml of *water*, make alkaline with *1 M sodium hydroxide*, extract with 5 ml of *chloroform*, filter through *anhydrous sodium sulphate* and evaporate the solvent in a current of nitrogen. The oily residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluphenazine hydrochloride IPRS* treated in the same manner or with the reference spectrum of fluphenazine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 258 nm and a less well-defined maximum at about 310 nm; absorbance at about 258 nm, between 0.63 and 0.70.

C. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*. Impregnate the dry plate by placing in a tank containing a shallow layer of a mixture of 36 volumes of *acetone*, 3 volumes of *formamide* and 1 volume of *2-phenoxyethanol*. Allow the impregnating solvent to ascend to the top, remove the plate from the tank and use it immediately.

Mobile phase. A mixture of 100 volumes of *light petroleum (40° to 60°)* saturated with *2-phenoxyethanol* and 2 volumes of *diethylamine*.

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of *methanol*.

Reference solution. A 0.2 per cent w/v solution of *fluphenazine hydrochloride IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, examine under ultraviolet light at 365 nm and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with *ethanolic sulphuric acid (20 per cent)* and observe the colour produced. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Mix about 5 mg with 45 mg of *heavy magnesium oxide* and ignite in a crucible until an almost white residue is obtained (usually less than 5 minutes). Allow to cool, add 1 ml of *water*, 0.05 ml of *dilute phenolphthalein solution* and about 1 ml of *2 M hydrochloric acid* to render the solution colourless and filter. To a freshly prepared mixture of 0.1 ml of *alizarin red S solution* and 0.1 ml of *zirconyl nitrate solution*, add 1 ml of the filtrate. Mix, allow to stand for 5 minutes and examine the colour of the solution as well as of a blank prepared in the same manner. The colour of the test solution is yellow and that of the blank is red.

E. It gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 1.9 to 2.3, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light and prepare the solutions immediately before use.

Solvent mixture. 4 volumes of *acetic acid* and 996 volumes of a 0.43 per cent w/v solution of *sodium octanesulphonate*.

Test solution. Dissolve 25 mg of the substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b). Dilute 5.0 ml of the solution to 25.0 ml with mobile phase A.

Reference solution (c). A 0.0001 per cent w/v solution of *fluphenazine impurity A IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 2 volumes of *acetic acid*, 150 volumes of *methanol*, 400 volumes of *acetonitrile* and 450 volumes of solvent mixture, ,

B. *methanol*,

- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm and 274 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
15	100	0
35	30	70
50	30	70
55	100	0

Name	Relative retention time	Correction factor
Fluphenazine impurity A[1]	0.2	—
Fluphenazine impurity B[2]	0.3	0.3
Fluphenazine (Retention time: about 19 minutes)	1.0	---
Fluphenazine impurity C[3]	2.2	0.6
Fluphenazine impurity D[4]	2.3	0.6

[1]fluphenazine sulphoxide,

[2]fluphenazine *S,S*-dioxide,

[3]2-[4-[3-[2',8-bis(trifluoromethyl)-10*H*-3,10'-biphenothiazin-10-yl]propyl]piperazin-1-yl]ethanol,

[4]10,10'-[piperazine-1,4-diylbis(propane-3,1-diyl)]bis[2-(trifluoromethyl)-10*H*-phenothiazine].

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and tailing factor is not more than 2.0.

Inject reference solution (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to fluphenazine impurity A at 274 nm is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent). The area of peak corresponding to fluphenazine impurity B at 274 nm is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of peak corresponding to fluphenazine impurities C and D at 260 nm is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak at 260 nm is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks other than fluphenazine impurities A and B at 260 nm and fluphenazine impurities A and B at 274 nm is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak at 260 nm with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh 0.25 g and dissolve in a mixture of 10 ml of *anhydrous formic acid* and 40 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02552 g of $C_{22}H_{26}F_3N_3OS,2HCl$.

Storage. Store protected from light.

Fluphenazine Hydrochloride Injection

Fluphenazine Hydrochloride Injection is a sterile solution of Fluphenazine Hydrochloride in Water for Injection.

Fluphenazine Hydrochloride Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of fluphenazine hydrochloride, $C_{22}H_{26}F_3N_3OS,2HCl$.

Usual strengths. 2.5 mg per ml; 10 mg per ml.

Description. A clear, colourless solution.

Identification

A. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*. Impregnate the dry plate by placing in a tank containing a shallow layer of a mixture of 36 volumes of *acetone*, 3 volumes of *formamide* and 1 volume of *2-phenoxyethanol*. Allow the impregnating solvent to ascend to the top, remove the plate from the tank and use it immediately.

Mobile phase. A mixture of 100 volumes of *light petroleum (40° to 60°)* saturated with *2-phenoxyethanol* and 2 volumes of *diethylamine*.

Test solution. Use a quantity of the injection containing 2 mg of Fluphenazine Hydrochloride and dilute it to 1 ml with *methanol*.

Reference solution. A 0.2 per cent w/v solution of *fluphenazine hydrochloride IPRS* in *methanol*.

After development, dry the plate in air, examine under ultraviolet light at 365 nm and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with *ethanolic sulphuric acid (20 per cent)* and observe the colour produced. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To a quantity of the injection containing 5 mg of Fluphenazine Hydrochloride add 2 ml of *sulphuric acid* and allow to stand for 5 minutes; an orange colour is produced.

Tests

pH (2.4.24). 4.8 to 5.2.

Related substances. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 80 volumes of *acetone*, 30 volumes of *cyclohexane* and 5 volumes of *strong ammonia solution*.

Test solution. Dilute a quantity of the injection containing about 20 mg of Fluphenazine Hydrochloride with sufficient *0.1 M methanolic sodium hydroxide* to make 10 ml.

Reference solution (a). Dilute 1 volume of the test solution to 50 volumes with *0.1 M methanolic sodium hydroxide*.

Reference solution (b). Dilute 1 volume of the test solution to 100 volumes with *0.1 M methanolic sodium hydroxide*.

Apply to the plate 50 µl of the test solution and 25 µl of reference solution (a) and (b). After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any spot remaining on the line of application.

Bacterial endotoxins (2.2.3). Not more than 166.7 Endotoxin Units per mg of fluphenazine hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *Carry out the following procedure protected from light.*

To a measured quantity of the injection containing 5 mg of Fluphenazine Hydrochloride add a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *ethanol* (*90 per cent*) to produce 50.0 ml. Dilute 10.0 ml of the solution to 100.0 ml with the acid-ethanol mixture and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of $C_{22}H_{26}F_3N_3OS,2HCl$ taking 620 as the specific absorbance at 258 nm.

Storage. Store protected from light.

Fluphenazine Tablets

Fluphenazine Hydrochloride Tablets

Fluphenazine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluphenazine hydrochloride, $C_{22}H_{26}F_3N_3OS,2HCl$. The tablets are coated.

Usual strengths. 1 mg; 2.5 mg; 5 mg.

Identification

A. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*. Impregnate the dry plate by placing in a tank containing a shallow layer of a mixture of 36 volumes of *acetone*, 3 volumes of *formamide* and 1 volume of *2-phenoxyethanol*. Allow the impregnating solvent to ascend to the top, remove the plate from the tank and use it immediately.

Mobile phase. A mixture of 100 volumes of *light petroleum (40° to 60°)* saturated with *2-phenoxyethanol* and 2 volumes of *diethylamine*.

Test solution. Shake a quantity of the powdered tablets with sufficient *methanol* to produce a solution containing 0.2 per cent w/v of Fluphenazine Hydrochloride, centrifuge and use the supernatant liquid.

Reference solution. A 0.2 per cent w/v solution of *fluphenazine hydrochloride IPRS* in *methanol*.

After development, dry the plate in air, examine under ultraviolet light at 365 nm and observe the fluorescence produced after about 2 minutes. Heat the plate at 120° for 20 minutes, cool, spray with *ethanolic sulphuric acid (20 per cent)* and observe the colour produced. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Extract a quantity of the powdered tablets containing 5 mg of Fluphenazine Hydrochloride with 5 ml of *acetone*, filter and evaporate the filtrate to dryness. Add 2 ml of *sulphuric acid* to the residue and allow to stand for 5 minutes; an orange colour is produced.

C. Extract a quantity of the powdered tablets containing 10 mg of Fluphenazine Hydrochloride with 10 ml of *ethanol* containing 0.2 per cent v/v of *strong ammonia solution* and evaporate the extract to dryness. Heat 0.5 ml of *chromic-sulphuric acid mixture* in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the residue and again heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of *0.01M hydrochloric acid*,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Buffer solution. 0.05 M monobasic potassium phosphate, adjusted to pH 2.5 with *orthophosphoric acid.*

Solvent mixture. 30 volumes of *acetonitrile* and 30 volumes of *methanol* and 40 volumes of the buffer solution.

Test solution. Dilute the filtrate with an equal volume of the mobile phase.

Reference solution. Dissolve a suitable quantity of *fluphenazine hydrochloride IPRS* in the dissolution medium and dilute with an equal volume of the mobile phase to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 12.5 cm × 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: 0.3 per cent w/v solution of *triethylamine* in the solvent mixture,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{26}F_3N_3OS$, 2HCl, in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{26}F_3N_3OS$, 2HCl.

Related substances. Determine in subdued light by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 80 volumes of *acetone*, 30 volumes of *cyclohexane* and 5 volumes of *strong ammonia solution.*

Test solution. Remove the coating from a suitable quantity of tablets; shake a quantity of the powdered tablet cores containing 20 mg of Fluphenazine Hydrochloride with 10 ml of *0.1 M methanolic sodium hydroxide* for 5 minutes, centrifuge and use the supernatant liquid.

Reference solution (a). Dilute 1 volume of the test solution to 50 volumes with *0.1 M methanolic sodium hydroxide.*

Reference solution (b). Dilute 1 volume of the test solution to 100 volumes with *0.1 M methanolic sodium hydroxide.*

Apply to the plate 50 µl of the test solution and 25 µl of reference solution (a) and (b). After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots is more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any spot remaining on the line of application.

Uniformity of content. Complies with the test stated under Tablets.

NOTE — Carry out the procedure protected from light.

Powder 1 tablet and dissolve the powder as completely as possible in a mixture of 99 volumes of *ethanol (80 per cent)* and 1 volume of *1 M hydrochloric acid.* Add sufficient of the acid-ethanol mixture to produce 100.0 ml and filter. Dilute suitably, if necessary with the acid-ethanol mixture and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of $C_{22}H_{26}F_3N_3OS$,2HCl taking 620 as the specific absorbance at 258 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Dissolution with the following modifications.

Test solution. Disperse 5 intact tablets in the solvent mixture, with intermittent shaking for 1 hour and sonicate until a fine suspension is obtained and dilute with the solvent mixture to obtain a solution containing 0.005 per cent w/v of Fluphenazine Hydrochloride.

Reference solution. A 0.005 per cent w/v solution of *fluphenazine hydrochloride IPRS* in the solvent mixture.

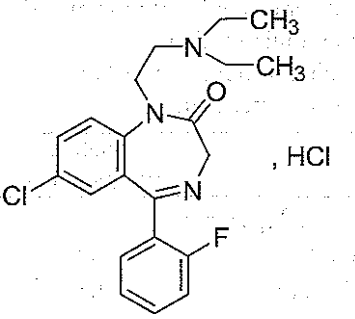
- mobile phase: 0.2 per cent w/v solution of *triethylamine* in the solvent mixture,
- flow rate: 1 ml per minute,
- injection volume: 25 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{26}F_3N_3OS$,2HCl in the tablets.

Storage. Store protected from light.

Flurazepam Hydrochloride



$C_{21}H_{23}ClFN_3O$,HCl Mol. Wt. 424.3

Flurazepam Hydrochloride is 7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one monohydrochloride.

Flurazepam Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{23}ClFN_3O,HCl$, calculated on the dried basis.

Category. Sedative; anxiolytic; anticonvulsant and skeletal muscle relaxant.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flurazepam hydrochloride IPRS* or with the reference spectrum of flurazepam hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 5.0 to 6.0, determined on 5.0 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg each of the substance under examination and *oxazepam* in 10 m of *acetonitrile* and dilute to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a 1.05 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 6.1 with 4.0 per cent w/v solution of *sodium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Flurazepam (Retention time: about 7 minutes)	1.0	—
Flurazepam impurity C[1]	1.5	0.65
Flurazepam impurity B[2]	1.9	0.61
Flurazepam impurity A[3]	2.4	—

[1]7-chloro-5-(2-fluorophenyl)-1-[(1*RS*)-1-hydroxyethyl]-1,3-dihydro-2H-1,4-benzodiazepin-2-one,

[2]7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one,

[3][5-chloro-2-[[2-(diethylamino)ethyl]amino]phenyl](2-fluorophenyl) methanone.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to flurazepam and oxazepam is not less than 4.5.

Inject reference solution (a) and the test solution. Run the chromatogram 6 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Fluorides (2.3.55). 0.1 g complies with the limit test for fluorides (500 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Dissolve 0.35 g in a mixture of 1.0 ml of *0.1 M hydrochloric acid* and 50 ml of *ethanol*. Titrate with *0.1 M sodium hydroxide*. Read the volume added between 2 points of inflection.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.04243 g of $C_{21}H_{24}Cl_2FN_3O$.

Storage. Store protected from light.

Flurazepam Capsules

Flurazepam Capsules contain Flurazepam Hydrochloride.

Flurazepam Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of flurazepam, $C_{21}H_{23}ClFN_3O$.

Usual strengths. 5 mg; 10 mg; 15 mg.

Identification

A. Shake a quantity of the capsules containing 0.15 g of Flurazepam with 3 ml of *chloroform* and filter. On the filtrate, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flurazepam hydrochloride IPRS* or with the reference spectrum of flurazepam hydrochloride.

B. When examined in the range 220 nm to 350 nm (2.4.7), the solution obtained in the Assay exhibits two maxima at 240 nm and 284 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2.5 volumes of *diethylamine* and 97.5 volumes of *ether*.

Solvent mixture. 2 volumes of *13.5 M ammonia* and 98 volumes of *methanol*.

Test solution. Disperse a quantity of the contents of capsules containing 0.1 g of flurazepam with 2 ml of the solvent mixture and centrifuge.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with the solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

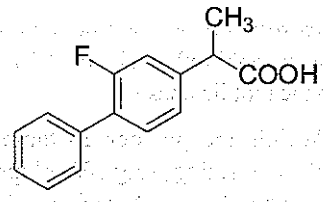
Other tests. Comply with the tests stated under Capsules.

Assay. *Carry out the following procedure protected from light.*

Disperse a quantity of the capsule contents containing 0.1 g of flurazepam in 150 ml of *1 M methanolic sulphuric acid* with the aid of ultrasound for 10 minutes and dilute to 250 ml with *1 M methanolic sulphuric acid*. Further dilute the solution with *1 M methanolic sulphuric acid* to obtain a solution containing 0.002 per cent w/v of flurazepam and measure the absorbance of the resulting solution at the maximum at 284 nm (2.4.7). Calculate the content of $C_{21}H_{23}ClFN_3O$ taking 319 as the value of specific absorbance at the maximum at 284 nm.

Labelling. The label states that the strength in terms of the equivalent amount of Flurazepam.

Flurbiprofen



$C_{15}H_{13}FO_2$ Mol. Wt. 244.3

Flurbiprofen is (*RS*)-2-(2-fluorobiphenyl-4-yl)propionic acid.

Flurbiprofen contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{15}H_{13}FO_2$, calculated on the dried basis.

Category. Antiinflammatory; analgesic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flurbiprofen IPRS* or with the reference spectrum of flurbiprofen.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorption maximum only at about 247 nm, about 0.8.

C. Heat 0.5 ml of *chromic-sulphuric acid mixture* in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 45 volumes of *acetonitrile* and 55 volumes of *water*.

Test solution (a). Dissolve 0.2 g of the substance under examination in 100.0 ml with solvent mixture.

Test solution (b). Dissolve 0.2 g of the substance under examination and 1 mg of *2-(biphenyl- 4-yl) propionic acid IPRS* in 100.0 ml of the solvent mixture.

Reference solution. A 0.001 per cent w/v solution of *2-(biphenyl-4-yl) propionic acid IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 35 volumes of *acetonitrile* and 5 volumes of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Adjust the sensitivity of the instrument so that with the reference solution the height of the peak due to 2-(biphenyl-4-yl)propionic acid is about 40 per cent of the full-scale deflection on the recorder.

In the chromatogram obtained with test solution (a) the area of any secondary peak is not greater than the area of the peak in the chromatogram obtained with the reference solution and the sum of the areas of any such peaks is not greater than

twice the area of the peak in the chromatogram obtained with the reference solution.

The test is not valid unless a peak due to 2-(biphenyl-4-yl) propionic acid appears immediately before the principal peak in the chromatogram obtained with test solution (b) and the height of the trough separating the two peaks is less than 4 per cent of the full-scale deflection on the chart paper.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying. Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Weigh 0.5 g, dissolve in 100 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02443 g of $C_{15}H_{13}FO_2$.

Flurbiprofen Tablets

Flurbiprofen Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of flurbiprofen, $C_{15}H_{13}FO_2$. The tablets are coated.

Usual strengths. 50 mg; 100 mg.

Identification

Extract a quantity of the powdered tablets containing 0.5 g of Flurbiprofen with 25 ml of *acetone*, filter, evaporate the filtrate to dryness with the aid of a current of air without heating and dry at 60° at a pressure of 2 kPa. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flurbiprofen IPRS* or with the reference spectrum of flurbiprofen.

B. Heat 0.5 ml of *chromic-sulphuric acid mixture* in a small test-tube in a water-bath for 5 minutes; the solution wets the sides of the tube readily and there is no greasiness. Add 2 or 3 mg of the substance under examination and heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.

Tests

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium. 900 ml of a buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* and 1.4 g of *sodium hydroxide* in 900 ml of *water*, adjusted to pH 7.2 with *5M sodium hydroxide* or *orthophosphoric acid* and dilute to 1000 ml with *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with medium, if necessary, at the maximum at about 247 nm (2.4.7). Calculate the content of $C_{15}H_{13}FO_2$ in the medium from the absorbance obtained from a solution of known concentration of *flurbiprofen IPRS* in dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{15}H_{13}FO_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.5 g of Flurbiprofen in 50 ml of *water*, add 200 ml of *acetonitrile*, mix and centrifuge. Use the supernatant liquid.

Reference solution. A 0.001 per cent w/v solution of *2-(biphenyl-4-yl)propionic acid IPRS* in the test solution.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 35 volumes of *acetonitrile* and 5 volumes of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Adjust the sensitivity of the instrument so that with the reference solution the height of the peak due to 2-(biphenyl-4-yl) propionic acid is about 40 per cent of the full-scale deflection on the recorder.

In the chromatogram obtained with the test solution the area of any secondary peak is not greater than the area of the peak in the chromatogram obtained with the reference solution and the sum of the areas of any such peaks is not greater than twice the area of the peak in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14),

Internal standard solution. A 0.008 per cent w/v solution of *acetophenone* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.3 g of Flurbiprofen in internal standard solution, with the aid of mechanical shaking for 15 minutes and dilute to 100.0 ml with internal standard solution. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. A 0.3 per cent w/v solution of *flurbiprofen IPRS* in internal standard solution. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 57 volumes of buffer solution prepared by dissolving 2.5 g of *sodium dihydrogen orthophosphate* in 1000 ml of *water*, and 43 volumes of *acetonitrile* and adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

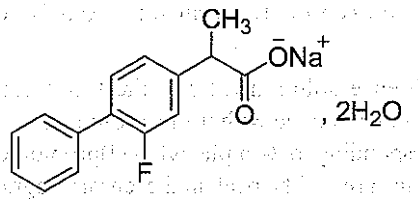
The relative retention time with reference to flurbiprofen for acetophenone is about 0.4.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to acetophenone and flurbiprofen is not less than 8.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{13}FO_2$ in the tablets.

Flurbiprofen Sodium



$C_{15}H_{12}FNaO_2,2H_2O$ Mol. Wt. 302.3

Flurbiprofen Sodium is Sodium (*RS*)-2-(2-fluorobiphenyl-4-yl) propionate dihydrate.

Flurbiprofen Sodium contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{15}H_{12}FNaO_2$, calculated on the dried basis.

Category. Analgesic; anti-inflammatory.

Description. A white to creamy-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flurbiprofen sodium IPRS* or with the reference spectrum of flurbiprofen sodium.

B. Heat 0.2 g over a flame until charred and then heat at 600° for 2 hours. The residue gives thse reactions of sodium salts (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *water* and 50 volumes of *methanol*.

Test solution. Dissolve 100 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.0002 per cent w/v solution of the substance under examination in the solvent mixture.

Reference solution (b). A 0.0005 per cent w/v solution of *2-(biphenyl-4-yl)propionic acid IPRS* in the solvent mixture.

Reference solution (c). A solution containing 0.0005 per cent w/v each of the substance under examination and *2-(biphenyl-4-yl)propionic acid IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 5 volumes of *glacial acetic acid*, 35 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the two principal peaks is not less than 1.5.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-(biphenyl-4-yl)propionic acid is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Heavy metals (2.3.13). 12 ml of a 20.0 per cent w/v solution in *methanol* complies with the limit test for Heavy metals, Method D (10 ppm), using 10.0 ml of *lead standard solution (2 ppm Pb)*.

Loss on drying (2.4.19). 11.3 per cent to 12.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at 60° at a pressure of 2 kPa for 18 hours.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *water* and 50 volumes of *methanol*.

Test solution. Dissolve 15 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). A 0.015 per cent w/v solution of *flurbiprofen sodium IPRS* in the solvent mixture.

Reference solution (b). A solution contanining 0.00075 per cent w/v each of the substance under examination and *2-(biphenyl-4-yl)propionic acid IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{12}FNaO_2$.

Flurbiprofen Eye Drops

Flurbiprofen Eye Drops is a sterile solution of Flurbiprofen Sodium in Purified Water.

Flurbiprofen Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of flurbiprofen sodium dihydrate, $C_{15}H_{12}FNaO_2.2H_2O$.

Usual strengths. 0.03 per cent w/v; 0.3 per cent w/v.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 25 volumes of *water* and 50 volumes of *methanol*.

Mobile phase. A mixture of 5 volumes of *propan-2-ol* and 95 volumes of *dichloromethane* as the mobile phase.

Test solution. Dilute a volume of eye drops to obtain a 0.01 per cent w/v solution of Flurbiprofen Sodium in the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *flurbiprofen sodium IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 6.0 to 7.0.

2-(biphenyl-4-yl)propionic acid. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *water* and 50 volumes of *methanol*.

Test solution. Dilute a volume of eye drops to obtain 0.03 per cent w/v solution of Flurbiprofen Sodium in the solvent mixture.

Reference solution (a). A 0.00015 per cent w/v solution of *2-(biphenyl- 4-yl)propionic acid IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0005 per cent w/v each of *flurbiprofen sodium IPRS* and *2-(biphenyl-4-yl)propionic acid IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 5 volumes of *glacial acetic acid*, 35 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 1.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-(biphenyl-4-yl)propionic acid is not more than the area of the peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *water* and 50 volumes of *methanol*.

Test solution. Dilute a volume of eye drops to obtain a 0.015 per cent w/v solution of Flurbiprofen Sodium in the solvent mixture.

Reference solution (a). A 0.015 per cent w/v solution of *flurbiprofen sodium IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.0005 per cent w/v each of *flurbiprofen sodium IPRS* and *2-(biphenyl-4-yl)propionic acid IPRS* in the solvent mixture.

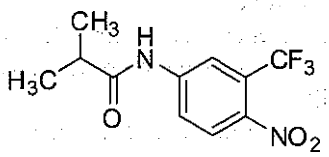
Use chromatographic system as described under test for 2-(Biphenyl-4- yl)propionic acid.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{12}FNaO_2.2H_2O$ in the eye drops.

Flutamide



$C_{11}H_{11}F_3N_2O_3$ Mol. Wt. 276.2

Flutamide is 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl] propanamide.

Flutamide contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{11}H_{11}F_3N_2O_3$, calculated on the dried basis.

Category. Antiandrogen.

Description. A pale yellow crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *flutamide IPRS* or with the reference spectrum of flutamide.

B. Melting point (2.4.21). About 112°.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 20.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b). Dissolve 2 mg of *flutamide IPRS* and 2 mg of *N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide IPRS (flutamide impurity C IPRS)* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *acetonitrile* and *water*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to flutamide impurity C and flutamide is not less than 10.5. The relative retention time with reference to flutamide for flutamide impurity C is about 0.72.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak corresponding to flutamide impurity C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° for 3 hours.

Assay. Weigh 25 mg, dissolve in *methanol* and dilute to 25.0 ml with the *methanol*. Dilute 2.0 ml of the solution to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 295 nm (2.4.7). Calculate the content of $C_{11}H_{11}F_3N_2O_3$ taking 295 as the specific absorbance at 295 nm.

Storage. Store protected from light.

Flutamide Capsules

Flutamide Capsules contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of flutamide, $C_{11}H_{11}F_3N_2O_3$.

Usual strength. 125 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel *GF254*.

Solvent mixture. 50 volumes of *chloroform* and 10 volumes of *methanol*.

Mobile phase. A mixture of 30 volumes of *chloroform* and 10 volumes of *ethyl acetate*.

Test solution. Dissolve the content of the capsules containing about 30 mg of Flutamide in 10.0 ml of the solvent mixture.

Reference solution. A 0.3 per cent w/v solution of *flutamide IPRS* in the solvent mixture.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 1000 ml of 2.0 per cent w/v solution of *sodium lauryl sulphate*,

Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 306 nm (2.4.7). Calculate the content of $C_{11}H_{11}F_3N_2O_3$ in the medium form a known concentration of *flutamide IPRS* prepared by initially dissolving in *methanol* and further diluting with the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{11}H_{11}F_3N_2O_3$.

Chromatographic purity. Determine by liquid chromatography (2.4.14), as described under Assay using the following modifications.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 10 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the secondary peak having relative retention time of 0.45 is not more than 0.2 per cent the area of the principal peak in the chromatogram obtained with the reference solution, the area of any other secondary peak is not more than 0.1 per cent the area of the principal peak in the chromatogram obtained with the reference solution and the sum of all the secondary peaks is not more than 0.3 per cent the area of the principal peak in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and of *water*.

Test solution. Mix the content of 20 Capsules. Disperse the content of the capsules containing about 125 mg of Flutamide in 250 ml of the solvent mixture, filter. Dilute 10.0 ml of the filtrate to 25 ml with *water*.

Reference solution. A 0.05 per cent w/v solution of *flutamide IPRS* in the solvent mixture. Dilute 10.0 ml of the solution to 25 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil),
- mobile phase: a mixture 55 volumes of *water* and 45 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{11}F_3N_2O_3$.

Storage. Store protected from light.

Flutamide Tablets

Flutamide Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of flutamide $C_{11}H_{11}F_3N_2O_3$.

Usual strength. 250 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 1000 ml of 2.0 per cent w/v solution of *sodium lauryl sulphate*,

Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate suitably diluted if necessary, with the dissolution medium at 306 nm (2.4.7). Calculate the

content of $C_{11}H_{11}F_3N_2O_3$ in the medium from the absorbance obtained from a solution of known concentration of *flutamide IPRS* prepared by dissolving in *methanol* and diluted with the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{11}H_{11}F_3N_2O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—All the solutions should be prepared under subdued light.

Test solution. Disperse a quantity of powdered tablets containing 100 mg of Flutamide in *acetonitrile* and dilute to 50.0 ml with *acetonitrile* and filter.

Reference solution. A 0.002 per cent w/v solution of *flutamide IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 25°,
- sample temperature: 5°,
- mobile phase: a mixture of 50 volumes of *water* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Other tests. Comply with the test stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—All the solution should be prepared under subdued light.

Test solution. Disperse a quantity of powdered tablets containing 100 mg of Flutamide in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with mobile phase.

Reference solution. A 0.02 per cent w/v solution of *flutamide IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *water* and 65 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

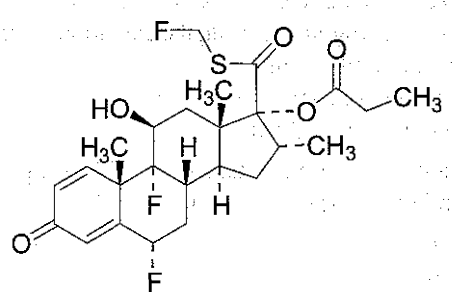
Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{11}F_3N_2O_3$ in the tablets.

Storage. Store protected from moisture not exceeding 30°.

Labelling. The label states the strength in terms of the amount of Flutamide.

Fluticasone Propionate



$C_{25}H_{31}F_3O_5S$ Mol. Wt. 500.6

Fluticasone Propionate is *S*-fluoromethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-17α-propionyloxy-3-oxoandrosta-1,4-diene-17β-carbothioate.

Fluticasone Propionate contains not less than 96.0 per cent and not more than 102.0 per cent of fluticasone, $C_{25}H_{31}F_3O_5S$, calculated on the anhydrous basis.

Category. Corticosteroid.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluticasone propionate IPRS* or with the reference spectrum of fluticasone propionate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +32.0° to +36.0°, determined in a 0.5 per cent w/v solution in *dichloromethane*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of mobile phase A and 50 volumes of mobile phase B.

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution. A 0.02 per cent w/v solution of *fluticasone propionate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. 0.05 per cent v/v *orthophosphoric acid* and 3.0 per cent v/v *methanol* in *acetonitrile*.
  B. 0.05 per cent v/v *orthophosphoric acid* and 3.0 per cent v/v *methanol* in *water*.
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	43	57
40	55	45
60	90	10
70	90	10
75	43	57
85	43	57

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 20,000 theoretical plates.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and sum of all the impurities is not more than 2.0 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.25 g, using *methanol* as solvent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. A 0.004 per cent w/v solution of *fluticasone propionate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of a buffer solution prepared by dissolving 1.15 g of *ammonium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of methanol,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{31}F_3O_5S$.

Storage. Store protected from light.

Fluticasone Cream

Fluticasone Cream contains Fluticasone Propionate in a suitable cream base.

Fluticasone Cream contains not less than 95.0 per cent and not more than 105.0 per cent of fluticasone propionate, $C_{25}H_{31}F_3O_5S$.

Usual strengths. 0.05 per cent w/w; 0.5 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane*.

Test solution. Transfer a quantity of the cream containing 1 mg of Fluticasone Propionate to a separating funnel, add 25 ml of *acetonitrile* and 25 ml of *n-hexane*, shake for 3 minutes and allow to separate. Filter the lower layer through an absorbent cotton plug, previously washed with *acetonitrile*, into a 50-ml graduated flask, repeat the extraction with one 5-ml and then one 2-ml quantity of *acetonitrile*, filter and add the extracts to the filtered layer; wash the absorbent cotton plug with 2 ml of *acetonitrile*, add the washings to the filtered layer and dilute the combined extracts to 50 ml with *acetonitrile*. Evaporate 4 ml of the resulting solution to

dryness using a rotary evaporator at a temperature of 40° and dissolve the residue in 0.2 ml of *acetonitrile*.

Reference solution. A 0.04 per cent w/v solution of *fluticasone propionate IPRS* in *acetonitrile*.

Apply to the plate 40 µl of each solution. Allow the plate to rise 12 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Transfer a quantity of the cream containing 1 mg of Fluticasone Propionate to a separating funnel, add 25 ml of *ethanol (65 per cent)*, stopper and shake until the cream is completely dispersed. Add 25 ml of *n-hexane*, shake for 3 minutes and allow to separate, filter the lower aqueous layer through an absorbent cotton plug, previously washed with *ethanol (65 per cent)*, into a graduated flask and repeat the extraction with one 5-ml and then one 2-ml quantity of *ethanol (65 per cent)*, filtering the aqueous ethanol extracts into the same graduated flask. Wash the absorbent cotton plug with *ethanol (65 per cent)*, collecting the washings in the flask and dilute the combined extracts to 50 ml with *ethanol (65 per cent)*.

Reference solution (a). A 0.002 per cent w/v solution of *fluticasone propionate IPRS* in *methanol (80 per cent)*.

Reference solution (b). A solution containing 0.0004 per cent w/v of *fluticasone* S-*methyl impurity IPRS* and 0.002 per cent w/v of *fluticasone propionate IPRS* in *methanol (80 per cent)*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of *0.01 M ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to fluticasone S-methyl impurity and fluticasone propionate is not less 1.6.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the cream.

Fluticasone Propionate Inhalation

Fluticasone Propionate Inhalation is a suspension of microfine Fluticasone Propionate in a suitable liquid filled in a suitable pressurized container. It may contain suitable pharmaceutical aids such as surfactants, stabilizing agents.

Fluticasone Propionate Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of fluticasone propionate, $C_{25}H_{31}F_3O_5S$, per inhalation by actuation of the valve.

Usual strengths. 50 µg per metered dose; 125 µg per metered dose; 250 µg per metered dose.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Other tests. Comply with the tests stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Determine by liquid chromatography (2.4.14).

Test solution. Prepare using the mobile phase as described under the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Reference solution (a). A 0.05 per cent w/v solution of *fluticasone propionate IPRS* in *acetonitrile*.

Reference solution (b). Dilute reference solution (a) with the mobile phase to obtain a solution containing 25 µg of fluticasone propionate per ml.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 1.3 g of *diammonium hydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.0

with *orthophosphoric acid* and 60 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 238 nm,
- inject volume: 200 µl or 100 µl.

Inject the reference solution (b). The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the solution and the content of $C_{25}H_{31}F_3O_5S$ delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of $C_{25}H_{31}F_3O_5S$ delivered per actuation of the valve meets the requirements.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the amount of active ingredient delivered per inhalation.

Fluticasone Propionate Powder for Inhalation

Fluticasone Propionate Powder for Inhalation consists of Fluticasone propionate in microfine powder either alone or admixed with Lactose in a pre-metered unit for use in a suitable powder inhaler.

Fluticasone Propionate Powder for Inhalation contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of fluticasone propionate, $C_{25}H_{31}F_3O_5S$ per unit dose.

Usual strengths. 50 mcg; 100 mcg; 250 mcg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Inhalation Preparations (Powders for Inhalation).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the mixed contents of 20 capsules in sufficient of the mobile phase to get a solution containing 25 µg of Fluticasone Propionate per ml.

Reference solution. A solution containing 0.5 mg of fluticasone propionate per ml prepared by dissolving 10 mg of *fluticasone propionate IPRS* in 10 ml *acetonitrile* and adding sufficient of the mobile phase to produce 20 ml and further dilute with mobile phase to obtain a solution containing 25 µg of Fluticasone Propionate per ml.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 40 volumes of a buffer solution prepared by dissolving 1.3 g of *diammonium hydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.0 with *orthophosphoric acid* and 60 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 200 µl or 100 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{31}F_3O_5S$ per unit.

Storage. Store protected from moisture, at temperature not exceeding 30°.

Labelling. The label states the quantity of active ingredient per pre-metered unit.

Fluticasone Nasal Spray

Fluticasone Nasal Spray is a suspension of Fluticasone Propionate in a suitable liquid in a container fitted with an appropriate nasal delivery system.

Fluticasone Nasal Spray contains not less than 80.0 per cent and not more than 120.0 per cent of fluticasone propionate, $C_{25}H_{31}F_3O_5S$ of the amount stated to be delivered by actuation of the valve.

Usual strengths. 50 µg; 100 µg; 125 µg.

NOTE — Carry out all the following procedures in the dark or under long-wavelength light at more than 420 nm and prepare solutions immediately before use and protect them from light.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane*.

Test solution. Dilute a quantity of the nasal spray containing 2 mg of Fluticasone Propionate to 5 ml with *acetonitrile*, shake for 3 minutes and filter.

Reference solution. A 0.04 per cent w/v solution of *fluticasone propionate IPRS* in *acetonitrile*.

Apply to the plate 40 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Dilute a quantity of the nasal spray containing 1 mg of Fluticasone Propionate to 5.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.02 per cent w/v of *fluticasone propionate IPRS* and 0.00004 per cent w/v of 6α,9-difluoro-17-[(methylsulfanyl)carbonyl]-11β-hydroxy-16α-methyl-3- oxoandrosta-1,4-dien-17α-yl propanoate (*fluticasone S-methyl impurity IPRS*) in the solvent mixture.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a solution containing 0.05 per cent v/v of *orthophosphoric acid* and 3.0 per cent v/v of *methanol* in *acetonitrile*,
  B. a solution containing 0.05 per cent v/v of *orthophosphoric acid* and 3.0 per cent v/v of *methanol* in *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent w/v)
0	43	57
40	55	45
60	90	10
70	90	10
75	43	57
85	43	57

Inject reference solution (b) and (c). The test is not valid unless the resolution between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is not less than 1.5 in the chromatogram obtained with reference solution (b) and the peak due to fluticasone propionate has a signal-to-noise ratio not less than 10 in the chromatogram obtained with reference solution (c).

Inject reference solution (a), (c) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of any such peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Other tests. Comply with the tests stated under Nasal Preparations.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. After priming the pump, discharge the container a sufficient number of times to obtain 1 mg of Fluticasone Propionate, add 25 ml of *ethanol (65 per cent)*, shake with the aid of ultrasound for 10 minutes and dilute to 50.0 ml with *ethanol (65 per cent)* and filter.

Reference solution (a). Dilute 2.0 ml of a 0.05 per cent w/v solution of *fluticasone propionate IPRS* in *methanol (80 per cent)* to 50 ml with *ethanol (65 per cent)*.

Reference solution (b). A solution containing 0.0004 per cent w/v of *fluticasone S-methyl impurity IPRS* and 0.002 per cent w/v of *fluticasone propionate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,

- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of *0.01 M ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to fluticasone *S*-methyl impurity and fluticasone propionate is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the nasal spray.

Labelling. The label states that the amount of active ingredient delivered by each actuation of the valve and the number of deliveries available from the container.

Fluticasone Ointment

Fluticasone Ointment contains Fluticasone Propionate in a suitable base.

Fluticasone Ointment contains not less than 95.0 per cent and not more than 105.0 per cent of fluticasone propionate, $C_{25}H_{31}F_3O_5S$.

Usual strength. 0.005 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 1 volume of *glacial acetic acid*, 8 volumes of *ethyl acetate* and 30 volumes of *dichloromethane*.

Test solution. Transfer a quantity of the ointment containing 0.1 mg of Fluticasone Propionate to a separating funnel, add 10 ml of *acetonitrile* and 50 ml of *n-hexane*, shake for 5 minutes and allow to separate. Filter the lower layer through an absorbent cotton plug previously washed with *acetonitrile*, extract the hexane layer with two 5-ml quantities of *acetonitrile*, filter each extract through the absorbent cotton plug and wash the absorbent cotton plug with 2 ml of *acetonitrile*. Add the washings to the combined filtrates, evaporate the resulting solution to dryness using a rotary evaporator at a temperature of 40° and dissolve the residue in 0.5 ml of *acetonitrile*.

Reference solution. A 0.02 per cent w/v solution of *fluticasone propionate IPRS* in *acetonitrile*.

Apply to the plate 20 µl of each solution. Allow the plate to rise 12 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Other tests. Comply with the tests stated under Ointment.

Assay. Determine by liquid chromatography (2.4.14).

NOTE— Protect the solutions from light.

Test solution. Transfer a quantity of the ointment containing 0.1 mg of Fluticasone Propionate to a separating funnel, add 45 ml of *n-hexane*, previously heated to 60° in a water-bath, stopper the funnel and shake until the ointment is dispersed, venting frequently. Wash the stopper and neck of the funnel with 5-ml of *n-hexane* collecting the washings in the funnel, allow the funnel to cool to room temperature, add 10 ml of *methanol (80 per cent)*, stopper, shake for 1 minute and allow to separate. Filter the lower aqueous layer through an absorbent cotton plug, previously washed with *methanol (80 per cent)*, into a graduated flask; repeat the extraction with two 5-ml quantities of *methanol (80 per cent)*, filtering the aqueous methanol extracts into the same graduated flask. Wash the absorbent cotton plug with 2 ml of *methanol (80 per cent)*, collecting the washings in the flask and dilute the extract to 25 ml with *methanol (80 per cent)*.

Reference solution (a). A 0.0004 per cent w/v solution of *fluticasone propionate IPRS* in *methanol (80 per cent)*.

Reference solution (b). A solution containing 0.0004 per cent w/v of *fluticasone* S-*methyl impurity IPRS* and 0.002 per cent w/v of *fluticasone propionate IPRS* in *methanol (80 per cent)*.

Chromatographic system

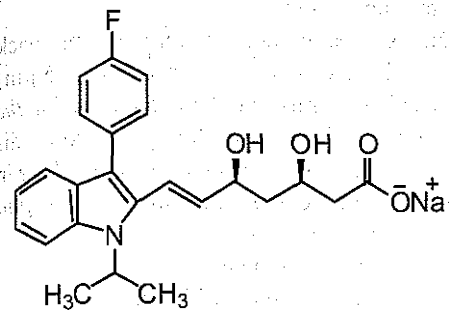
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 15 volumes of *acetonitrile*, 35 volumes of *0.01 M ammonium dihydrogen orthophosphate* previously adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to fluticasone *S*-methyl impurity and fluticasone propionate is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{25}H_{31}F_3O_5S$ in the ointment.

Fluvastatin Sodium



$C_{24}H_{25}FNNaO_4$ Mol. Wt. 433.5

Fluvastatin Sodium is Sodium (3*R**,5*S**,6*E*)-7-[3-(4-fluorophenyl)-1-(propan-2-yl)-1*H*-indol-2-yl]-3,5-dihydroxy-6-heptenoate.

Fluvastatin Sodium contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{24}H_{25}FNNaO_4$, calculated on the dried basis.

Category. Antihyperlipidemic.

Description. A white or almost white, or pale yellow to pale reddish-yellow, very hygroscopic, crystalline powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fluvastatin sodium IPRS* or with the reference spectrum of fluvastatin sodium.

B. A 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) gives reaction (A) of sodium (2.3.1).

Tests

pH (2.4.24). 8.0 to 10.0, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light.

Test solution. Dissolve 25 mg of the substance under examination in 20 ml of mobile phase B and dilute to 50.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with mobile phase A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 50.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. add 20 ml of a 25 per cent w/v solution of *tetramethylammonium hydroxide* to 880 ml of *water*, adjusted to pH 7.2 with *orthophosphoric acid*, mix with 100 ml of a mixture of 40 volumes of *acetonitrile* and 60 volumes of *methanol*,

  B. add 20 ml of a 25 per cent w/v solution of *tetramethylammonium hydroxide* to 80 ml of *water*, adjusted to pH 7.2 with *orthophosphoric acid* mix with 900 ml of a mixture of 40 volumes of *acetonitrile* and 60 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer at 305 nm and at 365 nm.
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
3	70	30
23	10	90
25	70	30
30	70	30

Name	Relative retention time
Fluvastatin (Retention time: about 14 minutes)	1.0
Fluvastatin impurity A[1]	1.05
Fluvastatin impurity D[2]	1.1
Fluvastatin impurity B[3]	1.6

[1](3*RS*,5*RS*,6*E*)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl]-3,5-dihydroxyhept-6-enoic acid,

[2] (6*E*)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl]-3-hydroxy-5-oxohept-6-enoic acid,

[3]1,1-dimethylethyl (3*R*,5*S*,6*E*)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl]-3,5-dihydroxyhept-6-enoate.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to fluvastatin impurity A at 305 nm is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent), the area of any peak due to fluvastatin impurity B at 305 nm is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any peak due to fluvastatin impurity D at 365 nm is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) at 305 nm (0.15 per cent), the area of any other secondary peak at 305 nm

is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent) and the sum of areas of all the secondary peaks at 305 nm is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).Ignore any peak at 305 nm with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). Dissolve 1.0 g in a mixture of 15 volumes of *water* and 85 volumes of *methanol* and dilute to 20 ml with the same solvent mixture. 12 ml of the solution complies with the limit test for heavy metals, Method D (20 ppm), using 10.0 ml of *lead standard solution (1 ppm Pb)* in the same solvent mixture.

Loss on drying (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.325 g in 50 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04335 g of $C_{24}H_{25}FNNaO_4$.

Storage. Store protected from light and moisture.

Fluvastatin Capsules

Fluvastatin Sodium Capsules

Fluvastatin Capsules contain fluvastatin sodium equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fluvastatin, $C_{24}H_{26}FNO_4$.

Usual strengths. 20 mg; 40 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve a weighted quantity of *fluvastatin sodium IPRS* in dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of buffer solution prepared by dissolving 1.534 g of *monobasic ammonium phosphate* in 800 ml of *water*, adjusted to pH 3.5 with *orthophosphoric acid* or *ammonium hydroxide*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{26}FNO_4$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{24}H_{26}FNO_4$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

NOTE— Protect all solutions from light.

Solvent mixture. 54 volumes of buffer solution pH 7.2 prepared as solution containing 40 ml of 25 per cent aqueous *tetramethylammonium hydroxide* in 1000 ml of *water* and adjusted to pH 7.2 with *orthophosphoric acid* and 46 volumes of a mixture of 3 volumes of *methanol* and 2 volumes of *acetonitrile*.

Test solution. Disperse the content of 10 capsules with 100 ml *methanol* with the aid of ultrasound for 45 minutes. Centrifuge of the solution at 4000 rpm for 20 minutes. Dilute a volume of the solution pH 7.2 containing about 20 mg of Fluvastatin to 50.0 ml with the solvent mixture.

Reference solution. A 0.042 per cent w/v solution of *fluvastatin sodium IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 87.5 volumes of buffer solution prepared by dissolving 40 ml of 25 per cent aqueous *tetramethylammonium hydroxide* in 1000 ml of *water*, adjusted to pH 7.2 with *orthophosphoric acid* and 12.5 volumes of a mixture of 3 volumes of *methanol* and 2 volumes of *acetonitrile*,

  B. a mixture of 87.5 volumes of a mixture of 3 volumes of *methanol* and 2 volumes of *acetonitrile* and 12.5 volumes of buffer solution pH 7.2,
- a gradient programme using the conditions given below,

- flow rate: 2 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume: 25 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	54	46
6	54	46
17	17	83
20	17	83
20.1	54	46
26.1	54	46

The relative retention time for fluvastatin anti-isomer with reference to fluvastatin is about 1.2.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.5 per cent.

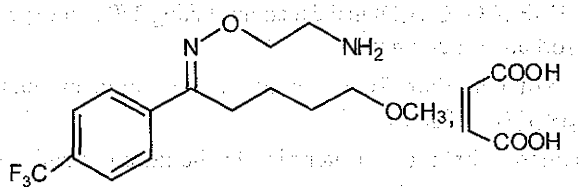
Inject the reference solution and the test solution.

1 mg of $C_{24}H_{25}FNNaO_4$ is equivalent to 0.95 mg of $C_{24}H_{26}FNO_4$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of fluvastatin.

Fluvoxamine Maleate



$C_{19}H_{25}F_3N_2O_6$ Mol. Wt. 434.4

Fluvoxamine Maleate is Fluvoxamine maleate is (*E*)-5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone *O*-(2-aminoethyl) oxime maleate.

Fluvoxamine Maleate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{19}H_{25}F_3N_2O_6$, calculated on the dried basis.

Category. Antidepressant.

Description. A white to off-white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with that of *fluvoxamine maleate IPRS* or with the reference spectrum of fluvoxamine maleate.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the test solution immediately before use.

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). A 0.2 per cent w/v solution of *fluvoxamine maleate IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 37 volumes of *acetonitrile* and 63 volumes of a buffer solution containing 0.11 per cent w/v of *potassium dihydrogen phosphate* and 0.19 per cent w/v of *sodium pentanesulphonate* in *water*, previously adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 234 nm,
- injection volume: 20 µl.

Name	Relative retention time
Maleic acid	0.15
Fluvoxamine impurity F[1]	0.5
Fluvoxamine impurity G[2]	0.5
Fluvoxamine impurity C[3]	0.6
Fluvoxamine impurity B[4]	0.8
Fluvoxamine (Retention time: about 15 minutes)	1.0
Fluvoxamine impurity A[5]	2.5
Fluvoxamine impurity D[6]	5.4

[1]*N*-[2-[[[(1*E*)-5-methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene] amino]oxy]ethyl]ethane-1,2-diamine,

[2](5*E*)-5-[(2-aminoethoxy)imino]-5-[4-(trifluoromethyl)phenyl] pentan-1-ol,

[3](2*RS*)-2-[[2-[[[(1*E*)-5-methoxy-1-[4-trifluoromethyl)phenyl] pentylidene] amino]oxy] ethyl]amino]butanedioic acid,

[4]2-[[[(1*Z*)-5-methoxy-1-[4-(trifluoromethyl)phenyl]pentylidene] amino]oxy]ethanamine,

[5]2-[[[(1*E*)-1-[4-(trifluoromethyl)phenyl]pentylidene]amino] oxy]ethanamine,

[6]5-methoxy-1-[4-(trifluoromethyl)phenyl]pentan-1-one.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram 6 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to fluvoxamine impurity B is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak due to fluvoxamine impurity C is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of the peak due to fluvoxamine impurity A is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any peak due to fluvoxamine impurity D is not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent) and the sum of areas of any peaks due to fluvoxamine impurities F and G is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) and the peak due to maleic acid.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 80° for 2 hours.

Assay. Dissolve 0.35 g in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out the blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04344 g of $C_{19}H_{25}F_3N_2O_6$.

Fluvoxamine Tablets

Fluvoxamine Maleate Tablets

Fluvoxamine Tablets contains not less than 92.5 per cent and not more than 105.0 per cent of the stated amount of fluvoxamine maleate, $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$.

Usual strengths. 25 mg; 50 mg; 100 mg.

Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Fluvoxamine Maleate with 10 ml of *acetonitrile* for 10 minutes, centrifuge and evaporate the supernatant liquid to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *fluvoxamine maleate IPRS* or with the reference spectrum of fluvoxamine maleate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 5 volumes of *13.5 M ammonia* and 95 volumes of *ethanol (95 per cent)*.

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Fluvoxamine Maleate with 5 ml of *methanol* for 10 minutes, centrifuge and use the supernatant liquid.

Reference solution. A 1.0 per cent w/v solution of *fluvoxamine maleate IPRS* in *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*,

Speed and time. 50 rpm and 20 minutes.

Withdraw a suitable volume of the medium and centrifuge. Measure the absorbance of the clear centrifuge liquid, suitably diluted if necessary with dissolution medium at 244 nm (2.4.7). Calculate the content of fluvoxamine maleate, $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$ in the medium taking 270 as the specific absorbance at 244 nm.

Q. Not less than 70 per cent of the stated amount of $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 0.25 g of Fluvoxamine Maleate with 125 ml of the mobile phase for 10 minutes and dilute to 250.0 ml with the mobile phase. Centrifuge and use the supernatant liquid.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Add 1.0 ml of *1 M hydrochloric acid* to 10.0 ml of the test solution and heat on a water-bath for 10 minutes.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octylsilane bonded to porous silica (5 µm),
- column temeperature: 35°,
- mobile phase: a mixture of 40 volumes of a solution containing 1.25 per cent w/v of *diammonium hydrogen*

orthophosphate and 0.275 per cent w/v of *sodium heptanesulphonate monohydrate* and 60 volumes of *methanol*, adjusted to pH 3.5 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b). The relative retention time with reference to fluvoxamine maleate (retention time: about 7 to 9 minutes) for addition product is about 0.65.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to 'addition product' is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore the peak due to maleic acid which elutes immediately after the solvent front and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.25 g of Fluvoxamine Maleate with 125 ml of the mobile phase for 10 minutes and dilute to 250.0 ml with the mobile phase, centrifuge. Dilute 1.0 ml the supernatant liquid to 10.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *fluvoxamine maleate IPRS* in the mobile phase.

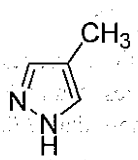
Use chromatographic system as described under Related substances.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{21}F_3N_2O_2,C_4H_4O_4$ in the tablets.

Storage. Store protected from light.

Fomepizole



$C_4H_6N_2$ Mol Wt. 82.1

Fomepizole is 4-methyl-1*H* pyrazole.

Fomepizole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_4H_6N_2$, calculated on the anhydrous basis.

Category. Antidote to methanol poisoning.

Description. A clear colourless liquid.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fomepizole IPRS* or with the reference spectrum of fomepizole.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Hydrazine hydrate. Not more than 0.001 per cent.

Determine by gas chromatography (2.4.13).

Test solution. Dissolve 1.0 g of the substance under examination in *acetone* and dilute to 10.0 ml with *acetone*.

Reference solution. Dissolve 1.25 µl *hydrazine hydrate IPRS* in *acetone* and dilute to 10.0 ml with *acetone*. Dilute 0.1 ml of the solution to 10.0 ml with *acetone*.

Chromatographic system
- a fused silica capillary column 30 m x 0.53 mm, packed with 6.0 per cent cyanopropyl phenyl and 94 per cent dimethyl polysiloxane (Such as DB-624)(film thickness 3.0 µm),
- temperature:
  column. 150° for 10 minutes, then raised at the rate of 50° per minute to 220°,hold for 3 minutes,
  inlet port at 200° and detector at 250°,
- flame ionisation detector,
- split ratio :25:1,
- flow rate: 1.2 ml per minute, using nitrogen as the carrier gas.
- injection volume: 1.0 µl.

Inject the reference solution and the test solution.

Calculate the content of hydrazine hydrate.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 20 µl of the substance under examination in *ethyl acetate* and dilute to 10.0 ml with *ethyl acetate*.

Chromatographic system
- a fused silica capillary column 30 m x 0.32 mm, packed with 5 per cent phenyl and 95 per cent methyl polysiloxane (Such as HP-5) (film thickness 0.25 µm),
- temperature:
  column. 70° for 3 minutes then raised to 250 ° at the rate of 20° per minute and hold for 15 minutes,

inlet port. at 250° and detector port at 270°,
- split ratio :50:1,
- flow rate:1.0 ml per minute, using nitrogen as the carrier gas,
- injection volume: 1.0 µl.

Inject the test solution. The area of any secondary peak at relative retention time 0.78 is not more than 0.1 per cent ,the area of any other secondary peak is not more than 0.2 per cent and the sum of the areas of all the secondary peaks is not more than 0.5 per cent, calculated by area normalization.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in *methanol* and diluted to100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with *methanol*.

Reference solution. A 0.01 per cent w/v solution of *fomepizole IPRS* in *methanol*.

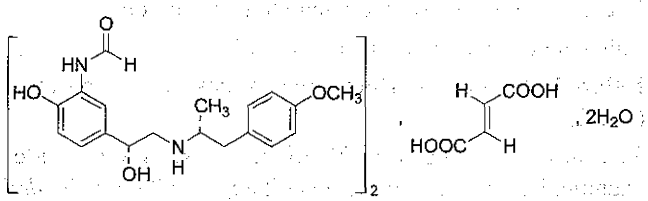
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of buffer solution prepared by dissolving 0.5ml of *trifluoroacetic acid* in 1000 ml of *water* and 10 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates , the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_4H_6N_2$.

Formoterol Fumarate Dihydrate



$(C_{19}H_{24}N_2O_4)_2, C_4H_4O_4, 2H_2O$ Mol. Wt. 840.9

Formoterol Fumarate Dihydrate is dihydrate salt of fumaric acid with (*RS*)-2′-hydroxy-5′-[(*RS*)-1-hydroxy-2-[[(*RS*)-*p*-methoxy-α-methylphenethyl]amino]ethyl]formanilide.

Formoterol Fumarate Dihydrate contains not less than 98.5 per cent and not more than 101.5 per cent of formoterol fumarate, $C_{42}H_{52}N_4O_{12}$, calculated on the anhydrous basis.

Category. Bronchodilator.

Description. A white or almost white or slightly yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *formoterol fumarate dihydrate IPRS* or with the reference spectrum of formoterol fumarate dihydrate.

Tests

pH (2.4.24). 5.5 to 6.5, determined in a 0.1 per cent w/v solution in *carbon dioxide-free water*.

Optical rotation (2.4.22). – 0.10° to + 0.10°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 84 volumes of a buffer solution prepared by dissolving 6.10 g of *sodium dihydrogen phosphate monohydrate* and 1.03 g of *disodium hydrogen phosphate dihydrate* in 1000 ml *water*, and 16 volumes of *acetonitrile*.

Test solution. Dissolve 20 mg of the substance under examination in 100 ml of the solvent mixture.

Reference solution. A 0.00004 per cent w/v solution of *formoterol fumarate IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with spherical octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *acetonitrile*,
  B. a buffer solution prepared by dissolving 3.73 g of *sodium dihydrogen phosphate monohydrate* and 0.35 g of *orthophosphoric acid* in 1000 ml of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	16	84
10	16	84
37	70	30
40	16	84
55	16	84

Inject the reference solution. The test is not valid unless the column efficiency in not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities is not more

than 1.0 per cent. Ignore the peak corresponding to fumaric acid.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.1 g.

Assay. Weigh 0.35 g and dissolve in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04024 g of $C_{42}H_{52}N_4O_{12}$.

Storage. Store protected from light and moisture.

Formoterol Fumarate and Budesonide Powder for Inhalation

Formoterol Fumarate and Budesonide Powder for Inhalation consists of Formoterol Fumarate and Budesonide in microfine powder either alone or admixed with Lactose in a pre-metered unit for use in a suitable powder inhaler.

Formoterol Fumarate and Budesonide Powder for Inhalation contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amounts of formoterol fumarate $C_{42}H_{52}N_4O_{12}$ and budesonide $C_{25}H_{34}O_6$ per pre-metered unit.

Usual Strengths. Formoterol Fumarate 6 µg and Budesonide 100 µg; Formoterol Fumarate 6 µg and Budesonide 200 µg; Formoterol Fumarate 6 µg and Budesonide 400 µg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

Tests

Other tests. Comply with the tests stated under the Inhalation Preparations (Powders for Inhalation).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the mixed contents of 20 capsules in sufficient of the mobile phase to get a solution containing 0.6 µg of Formoterol Fumarate per ml.

Reference solution (a). A 0.06 mg per ml solution of *formoterol fumarate IPRS* prepared by initially dissolving in 5 ml *acetonitrile* and then making up to volume with the mobile phase.

Reference solution (b). A 0.2 mg per ml solution of *budesonide IPRS* prepared by initially dissolving in 5 ml *acetonitrile* and then making up to volume with the mobile phase.

Reference solution (c). Dilute suitable volumes of reference solution (a) and reference solution (b) with the mobile phase to obtain a solution containing 0.6 µg of Formoterol Fumarate and 40 µg per ml of Budesonide per ml.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 0.138 g *sodium dihydrogen orthophosphate monohydrate* and 0.122 g of *decane sulphonic acid sodium salt* in 100 ml of *water* and adjusted to pH 3.0 with *orthophosphoric acid*, and 35 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 200 µl.

Inject reference solution (c). The order of elution is formoterol fumarate, budesonide epimer B and epimer A. The test is not valid unless the column efficiency determined from the formoterol fumarate and both the epimer peaks of Budesonide is not less than 1800 and 4000 theoretical plates respectively, the resolution between budesonide epimer peaks is not less than 1.5 and the relative standard deviation for formoterol fumarate and sum of peaks of budesonide epimer A and epimer B in replicate injections is not more than 2.0 per cent.

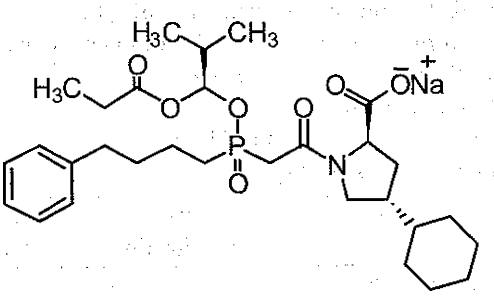
Inject reference solution (c) and the test solution.

Calculate the contents of $C_{42}H_{52}N_4O_{12}$ and $C_{25}H_{34}O_6$ per unit.

Storage. Store protected from moisture, at temperature not exceeding 30°.

Labelling. The label states the quantities of active ingredients per pre-metered unit.

Fosinopril Sodium



$C_{30}H_{45}NNaO_7P$ Mol. Wt. 585.7

Fosinopril Sodium is (4*S*)-4-Cyclohexyl-1-{[(*RS*)-2-methyl-1-(propionyloxy)propoxy]-(4-phenylbutyl)phosphinylacetyl}-*L*-proline sodium salt.

Fosinopril Sodium contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{30}H_{45}NNaO_7P$, calculated on the anhydrous basis.

Category. Antihypertensive.

Description. A white or almost white, crystalline powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fosinopril sodium IPRS* or with the reference spectrum of fosinopril sodium.

B. It gives reaction (A) of sodium salts (2.3.1).

Tests

Specific optical rotation (2.4.22). – 6.7° to – 4.7°, determined in 2.0 per cent w/v solution in *methanol.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.004 per cent w/v each of *fosinopril IPRS, fosinopril impurity A IPRS, fosinopril impurity B IPRS, fosinopril impurity I IPRS* and *fosinopril impurity K IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm packed with silica (5 µm),
- column temperature: 33°,
- mobile phase: a mixture of 0.5 volume of *orthophosphoric acid*, 3.5 volumes of *water* and 1000 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Fosinopril impurity K[1]	0.3	—
Fosinopril impurity I[2]	0.5	1.3
Fosinopril impurity B[3]	0.7	—
Fosinopril impurity E[4]	0.7	—
Fosinopril impurity H[5]	0.7	—
Fosinopril (Retention time: about 5 minutes)	1.0	—
Fosinopril impurity A[6]	2.0	—

[1](2*S*,4*S*)-4-cyclohexyl-1-(2,2-dimethyl-1-oxopropyl)pyrrolidine-2-carboxylic acid,

[2][(*RS*)-[(1*SR*)-2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl) phosphoryl]acetic acid,

[3](2*RS*,4*RS*)-4-cyclohexyl-1-[[(*RS*)-[(1*SR*)-2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphoryl]acetyl]pyrrolidine-2-carboxylic acid,

[4](2*S*,4*S*)-1-[[(*R*)-[(1*S*)-2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphoryl]acetyl]-4-phenylpyrrolidine-2-carboxylic acid,

[5](2*R*,4*S*)-4-cyclohexyl-1-[[(*R*)-[(1*S*)-2-methyl-1-(1-oxopropoxy) propoxy] (4-phenylbutyl)phosphoryl]acetyl]pyrrolidine-2-carboxylic acid,

[6](2*S*,4*S*)-4-cyclohexyl-1-[[(*R*)-hydroxy(4-phenylbutyl) phosphoryl]acetyl]-pyrrolidine-2-carboxylic acid.

Inject reference solution (a) and (c). The test is not valid unless the resolution between the peaks corresponding to fosinopril impurity B and fosinopril is not less than 2.0 in the chromatogram obtained with reference solution (a). In the chromatogram obtained with reference solution (c) signal-to-noise ratio for the principal peak is not less than 40.

Inject reference solution (b) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the area of peaks corresponding to fosinopril impurities B, E and H is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any peak corresponding to fosinopril impurity A is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak corresponding to fosinopril impurities I and K is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of any other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Impurities C and D. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.005 per cent w/v of *fosinopril IPRS* and *fosinopril impurity C IPRS* in the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c). A 0.005 per cent w/v solution of *fosinopril impurity D IPRS* in the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with strongly basic anion exchange resin (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 0.2 volume of *orthophosphoric acid*, 1.5 volumes of *water* and 400 volumes of *acetonitrile*,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 214 nm,
- injection volum: 20 µl.

Name	Relative retention time
Fosinopril (Retention time: about 10 minutes)	1.0
Fosinopril impurity C[1]	1.2
Fosinopril impurity D[2]	1.3

[1]mixture of (2*S*,4*S*)-4-cyclohexyl-1-[[(*S*)-[(1*S*)-2-methyl-1-(1-oxopropoxy)-propoxy](4-phenylbutyl)phosphoryl]acetyl]pyrrolidine-2-carboxylic acid and (2*S*,4*S*)-4-cyclohexyl-1-[[(*R*)-[(1*R*)-2-methyl-1-(1-oxopropoxy)propoxy] (4-phenylbutyl)phosphoryl] acetyl] pyrrolidine-2-carboxylic acid,

[2](2*S*,4*R*)-4-cyclohexyl-1-[[(*R*)-[(1*S*)-2-methyl-1-(1-oxopropoxy) propoxy] (4-phenylbutyl)phosphoryl]acetyl]pyrrolidine-2-carboxylic acid.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to fosinopril and impurity C is not less than 1.5.

Inject reference solution (b), (c) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to fosinopril impurity C is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any peak corresponding to fosinopril impurity D is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent).

Impurities E and F. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 20.0 ml with the mobile phase.

Reference solution (c). Dissolve the contents of a vial of *fosinopril impurity mixture IPRS* (fosinopril impurity E and F) in 1.0 ml of reference solution (a).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with phenylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 44 volumes of 0.2 per cent v/v of *orthophosphoric acid* and 56 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volum: 20 µl.

Name	Relative retention time	Correction factor
Fosinopril impurity E[1]	0.8	0.7
Fosinopril impurity F[2]	0.9	---
Fosinopril (Retention time: about 8 minutes)	1.0	---

[1](2*S*,4*S*)-1-[[(*R*)-[(1*S*)-2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphoryl]acetyl]-4-phenylpyrrolidine-2-carboxylic acid,

[2](2*S*,4*S*)-4-cyclohexyl-1-[[(*R*)-(4-phenylbutyl)[(1*S*)-1-(1-oxopropoxy)-propoxy]phosphoryl]acetyl]pyrrolidine-2-carboxylic acid.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to fosinopril and fosinopril impurity F is not less than 1.5.

Inject reference solution (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to fosinopril impurity F is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any peak corresponding to fosinopril impurity E is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent).

2-Ethylhexanoic acid (2.3.51). Not more than 0.2 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method C (20 ppm).

Water (2.3.43). Not more than 0.2 per cent, determined on 1.0 g.

Assay. Dissolve 0.45 g in 50 ml of *water*. Titrate with *0.1 M hydrochloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.05857 g of $C_{30}H_{45}NNaO_7P$.

Fosinopril Sodium Tablets

Fosinopril Sodium Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fosinopril sodium, $C_{30}H_{45}NNaO_7P$.

Usual strengths. 10 mg; 20 mg.

Identification

A. Transfer a quantity of the powdered tablets containing 25 mg of Fosinopril Sodium in 100 ml beaker, add 40 ml of *water*, heat at 25° for 5 minutes with stirring, filter and centrifuge at 2500 rpm for 30 minutes. Adjusted to pH 3.0 with *orthophosphoric acid* and pass through a fritted-disc funnel. Dissolve the precipitate by passing *chloroform* through filter, and evaporate the chloroform solution to dryness in air. On the oily residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fosinopril sodium IPRS*, treated in the same manner or with the reference spectrum of fosinopril.

B. In the Assay, the retention time of principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). Dissolve a quantity of *fosinopril sodium IPRS* in minimum amount of *methanol* and dilute with the dissolution medium to produce a solution of a known concentration similar to the expected concentration of the test solution.

Reference solution (b). A solution containing 0.002 per cent w/v each *fosinopril sodium IPRS* and *fosinopril impurity G IPRS ((4-phenylbutyl)phosphinylacetic acid disodium IPRS)* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 36 volumes of 0.2 per cent w/v of *orthophosphoric acid* and 64 volumes of *acetonitrile*,
- flow rate: 3 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to fosinopril sodium and fosinopril impurity G is not less than 1.7 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{30}H_{45}NNaO_7P$ in the tablet.

Q. Not less than 80 per cent of the stated amount of $C_{30}H_{465}NNaO_7P$.

Impurity A. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Reference solution. A 0.1 per cent w/v solution of *fosinopril impurity A IPRS* in *methanol* and dilute with the solvent mixture to obtain a solution containing 0.00025 per cent w/v of fosinopril impurity A.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to fosinopril impurity A is not more than 4.0 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *0.2 M urea solution* and 20 volumes of *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 50 mg of Fosinopril Sodium in 40.0 ml of the solvent mixture with the aid of ultrasound for 40 minutes. Dilute to 500.0 ml with the solvent mixture, mix, and centrifuge.

Reference solution (a). A 0.01 per cent w/v solution of *fosinopril sodium IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.007 per cent w/v of *fosinopril sodium IPRS* and 0.003 per cent w/v of *fosinopril impurity A IPRS((4S)-4-cyclohexyl-[(4-phenyl butyl)phosphinyl]acetyl-L-proline IPRS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 30 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 22 volumes of *0.2 per cent orthophosphoric acid* and 78 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

The relative retention time with reference to fosinopril for fosinopril impurity A is about 0.4.

Inject reference solution (b). The test is not valid unless the the resolution between the peaks due to fosinopril sodium and fosinopril impurity A is not less than 2.0 and the relative

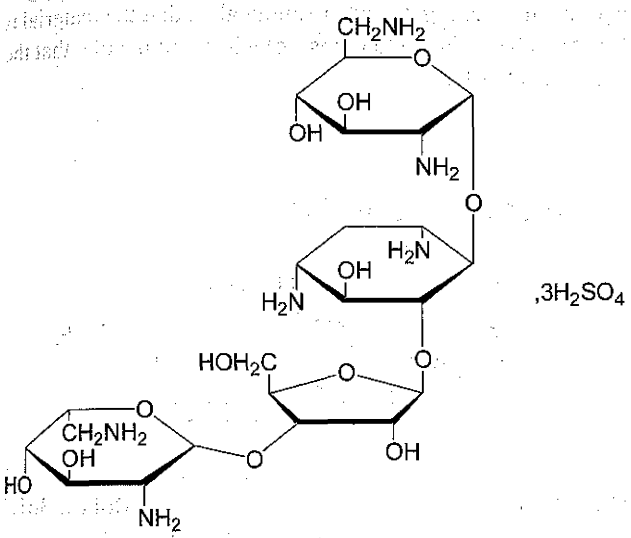
standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{30}H_{45}NNaO_7P$ in the tablets.

Storage. Store protected from moisture.

Framycetin Sulphate



$C_{23}H_{46}N_6O_{13},3H_2SO_4$ Mol. Wt. 908.9

Framycetin Sulphate is 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B) sulphate. The base is produced by the growth of selected strains of *Streptomyces fradiae* or *Streptomyces decaris* or by any other means.

Framycetin Sulphate contains an amount of framycetin sulphate equivalent to not less than 630 IU of neomycin B per mg, calculated on the dried basis.

Category. Antibacterial (topical).

Description. A white or yellowish-white powder; hygroscopic.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate in the following manner. Mix 0.3 g of *carbomer* with 240 ml of *water*, allow to stand for 1 hour with moderate shaking, adjusted to pH 7 by the gradual addition, with shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Spread a uniform layer of the suspension 0.75 mm thick, heat at 110° for 1 hour and allow to cool. Use the plate immediately.

Mobile phase. A 10 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. A 0.1 per cent w/v solution of the substance under examination.

Reference solution (a). A 0.1 per cent w/v solution of *framycetin sulphate IPRS*.

Reference solution (b). A solution containing 0.1 per cent w/v each of *framycetin sulphate IPRS*, *kanamycin sulphate IPRS* and *streptomycin sulphate IPRS*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air, spray with a mixture of equal volumes of a 46 per cent w/v solution of *sulphuric acid* and of a 0.2 per cent w/v solution of *1,3-naphthalenediol* in *ethanol (95 per cent)* and heat at 150° for about 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 10 mg in 5 ml of *water*, add 0.1 ml of *pyridine* and 2 ml of a 0.1 per cent w/v solution of *ninhydrin* and heat in a water-bath at 65° to 70° for 10 minutes; an intense violet colour is produced.

C. A 5 per cent w/v solution gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 6.0 to 7.0, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). +52.0° to +55.5°, determined at 20° in a 10.0 per cent w/v solution.

Neamine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 30 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *dichloromethane*.

Test solution. Dissolve 0.25 g of the substance under examination in *water* and dilute to 10 ml with *water*.

Reference solution. Dissolve 0.5 mg of *neamine IPRS* in 2 ml of *water*.

Apply 5 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate at 105° for 10 minutes. Spray it with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Spray the plate again with the same reagent and heat at 110° for 15 minutes. Any band corresponding to neamine in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with the reference solution (1 per cent).

Neomycin C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel of a suitable grade.

Mobile phase. A mixture of 80 volumes of a 20 per cent w/v solution of *sodium chloride* and 20 volumes of *methanol.*

Test solution. Dissolve 40 mg of the substance under examination in *water* and dilute to 5 ml with *water.*

Reference solution (a). Dissolve 40 mg of *framycetin sulphate IPRS* in *water* and dilute to 5 ml with *water.*

Reference solution (b). Dissolve 30 mg of *framycetin sulphate IPRS* in *water* and dilute to 25 ml with *water.* Dilute 5 ml of the solution to 25 ml with *water.*

Reference solution (c). Dissolve 40 mg of *neomycin sulphate IPRS* in *water* and dilute to 5 ml with *water.*

Apply 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 100° to 105° for 10 minutes. In the chromatogram obtained with the test solution, the principal band corresponds to the principal band in the chromatogram obtained with reference solution (a) and the band for neomycin C with Rf value slightly less than that of the principal band is not more intense than the band in the chromatogram obtained with reference solution (b) (3 per cent).

The test is not valid unless in the chromatogram obtained with reference solution (c), a band appears with R_f value slightly less than that of the principal band.

Sulphate. 27.0 to 31.0 per cent of SO_4, calculated on the dried basis, determined by the following method. Weigh 0.25 g, dissolve in 100 ml of *water,* adjusted to pH 11 with *strong ammonia solution* and add 10.0 ml of *0.1 M barium chloride.* Titrate with *0.1 M disodium edetate* using 0.5 mg of *metalphthalein* as indicator; add 50 ml of *ethanol (95 per cent)* when the colour of the solution begins to change and continue the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of SO_4.

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics (2.2.10), and express the results in µg of neomycin B per mg.

Framycetin Sulphate intended for administration into internal body cavities without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.3 Endotoxin Units per mg.

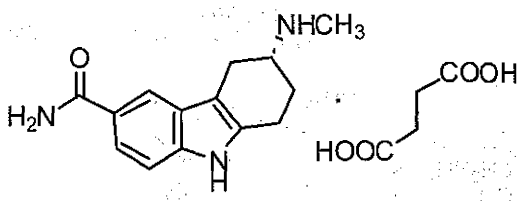
Framycetin Sulphate intended for administration into internal body cavities without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture at a temperature not exceeding 30°. If the material is sterile, the container should be tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the strength in terms of µg of neomycin B per mg; (b) where applicable, that the material is free from bacterial endotoxins; (c) where applicable, that the material is sterile.

Frovatriptan Succinate



$C_{18}H_{25}N_3O_5$ Mol wt. 361.4

Frovatriptan Succinate is (*R*)-5,6,7,8-Tetrahydro-6-methylaminocarbazole-3-carboxamide succinate.

Frovatriptan Succinate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{25}N_3O_5$, calculated on the anhydrous basis.

Category. Antimigraine.

Description. A white or off white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *frovatriptan succinate IPRS* or with the reference spectrum of frovatriptan succinate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of *water,* 5 volumes of *methanol* and 5 volumes of *acetonitrile.*

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50 ml with the solvent mixture .

Reference solution. A 0.00025 per cent w/v solution of *frovatriptan Succinate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *0.05 M ammonium acetate* in *water*, B. a mixture of 50 volumes of *acetonitrile* and 50.0 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
30	90	10
45	60	40
50	60	40
52	90	10
60	90	10

Inject the reference solution .The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of the areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalization.

Succinic acid. Dissolve 0.2 g in 50.0 ml of *water*. Titrate with *0.1 M sodium hydroxide*, using *phenolphthalein* as an indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0011809 g of succinic acid.

Water (2.3.43). Not more than 7.0 per cent , determined on 0.2 g.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of *water*, 5 volumes of *methanol* and 5 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50 ml with the solvent mixture . Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *frovatriptan succinate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of *0.05 M ammonium acetate* in *water* and 15 volumes of a mixture of 50 volumes of *acetonitrile* and 50 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 10 µl.

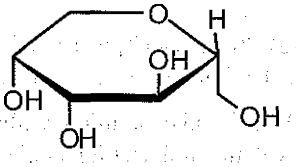
Inject the reference solution .The test is not valid unless the column efficiency is not less than 3000 theoretical plates , the tailing factor is not more than 2.0. and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{25}N_3O_5$.

Fructose

D-Fructose



$C_6H_{12}O_6$ Mol. Wt. 180.2

Fructose is D-(-)-fructopyranose.

Category. Nutrient; fluid replenisher.

Description. A white, crystalline powder with a very sweet taste.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with fructose *IPRS* or with the reference spectrum of fructose.

B. Dissolve 0.1 g in 10 ml of *water*, add 3 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

C. Dissolve 5 g in *water* and dilute to 10 ml with the same solvent. To 0.5 ml of the solution add 0.2 g of *resorcinol* and 9 ml of *dilute hydrochloric acid* and heat on a water-bath for 2 minutes; a red colour is produced.

Tests

Appearance of solution. Dissolve 5.0 g in *water* and dilute to 10 ml with the same solvent. The solution is clear (2.4.1). Add 10 ml of *water*. The solution is colourless (2.4.1).

Specific optical rotation (2.4.22). – 93.5° to – 91.0°, calculated on the anhydrous basis and determined on a solution prepared by dissolving 10.0 g in 80 ml of *water*, adding 0.2 ml of *5 M ammonia*, mixing well, allowing to stand for 30 minutes and diluting to 100.0 ml with *water*.

Acidity or alkalinity. Dissolve 6.0 g in 25 ml of *carbon dioxide-free water* and add 0.3 ml of *phenolphthalein solution*. The solution is colourless. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

5-Hydroxymethylfurfural and related compounds. To 5 ml of solution A add 5 ml of *water* and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.32).

Arsenic (2.3.10). Dissolve 10.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). A solution of 4.0 g in 10 ml of *water*, 2 ml of *dilute acetic acid* and sufficient *water* to produce 25.0 ml complies with the limit test for heavy metals, Method A (5 ppm).

Chlorides (2.3.12). 20 ml of a 10 per cent w/v solution (solution A) complies with the limit test for chlorides (125 ppm).

Sulphates (2.3.17). 7.5 ml of solution A diluted to 15 ml with *water* complies with the limit test for sulphates (200 ppm).

Foreign sugars. Dissolve 5.0 g in *water* and dilute to 10 ml with the same solvent. To 1 ml of the solution add 9 ml of *ethanol (95 per cent)*. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of the initial solution and 9 ml of *water*.

Barium. To 10 ml of solution A add 1 ml of *1 M sulphuric acid*. Examine exactly after 1 hour; any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution A and 1 ml of *water*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Fructose intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml of a 5 per cent w/v solution in Water for Injections.

Storage. Store protected from moisture.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Fructose Injection

Fructose Intravenous Infusion

Fructose Injection is a sterile solution of Fructose in Water for Injections.

Fructose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of fructose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Usual strength. 10 per cent w/v.

Description. A clear, colourless solution.

Identification

A. The solution prepared as directed in the Assay is laevorotatory.

B. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

Tests

pH (2.4.24). 3.0 to 6.0, determined in a solution diluted, if necessary, with Water for Injections to contain not more than 5.0 per cent w/v of Fructose and to which 0.30 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Fructose to 500.0 ml with *water* and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.50.

Heavy metals (2.3.13). A volume of the injection containing 4.0 g of fructose that has been evaporated to a volume of about 10 ml, cooled and diluted to 25 ml with *water* complies with the limit test for heavy metals, Method A (5 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Units per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume containing about 5.0 g of Fructose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube

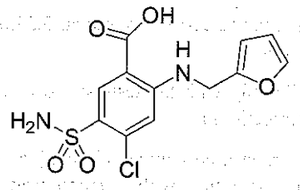
(2.4.22). The observed rotation in degrees multiplied by 0.5427 represents the weight, in g, of fructose, $C_6H_{12}O_6$, in the volume taken for Assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the strength as the percentage w/v of fructose, $C_6H_{12}O_6$; (2) that the injection should not be used if it contains visible particles.

Frusemide

Furosemide



$C_{12}H_{11}ClN_2O_5S$ Mol. Wt. 330.7

Frusemide is 4-chloro-*N*-furfuryl-5-sulphamoylanthranilic acid.

Frusemide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{12}H_{11}ClN_2O_5S$, calculated on the dried basis.

Category. Diuretic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *frusemide IPRS* or with the reference spectrum of frusemide.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.1 M sodium hydroxide* shows three absorption maxima at about 228 nm, 271 nm and 333 nm. The ratio of the absorbance at the maximum at about 271 nm to that at the maximum at about 228 nm is 0.52 to 0.57.

C. Dissolve about 5 mg in 10 ml of *methanol*. Transfer 1 ml of the solution to a flask, add 10 ml of *dilute hydrochloric acid* and boil under a reflux condenser on a water-bath for 15 minutes. Cool, add 15 ml of *1 M sodium hydroxide* and 5 ml of a 0.1 per cent w/v solution of *sodium nitrite*. Allow to stand for 3 minutes, add 5 ml of a 0.5 per cent w/v solution of *ammonium sulphamate*, mix and add 5 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*; a red-violet colour is produced.

Tests

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides (2.3.12). Shake 1 g with 40 ml of *water* for 5 minutes and filter. The filtrate complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). Shake 1.0 g with a mixture of 30 ml of *distilled water* and 0.2 ml of *5 M acetic acid* for 5 minutes and filter. 15 ml of the filtrate complies with the limit test for sulphates (300 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

Note—Prepare the solutions immediately before use.

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of solution prepared by dissolving 0.2 g of *potassium dihydrogen phosphate* and 0.25 g of *cetrimide* in 70 ml of *water* adjusted to pH 7.0 with *ammonia* and 30 volumes of *propanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 20 µl.

Inject the reference solution. Adjust the sensitivity so that the height of the peak is not less than 20 per cent of the full scale of the recorder.

Inject the reference solution and the test solution. Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area any secondary peak is not more than 0.25 times the area of the peak obtained with the reference solution (0.25 per cent) and sum of areas of all the secondary peaks is not more than 0.5 times the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.5 g, dissolve in 40 ml of *dimethylformamide* and titrate with *0.1 M sodium hydroxide* using *bromothymol blue solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03307 g of $C_{12}H_{11}ClN_2O_5S$.

Storage. Store protected from light.

Frusemide Injection

Furosemide Injection

Frusemide Injection is a sterile solution of Frusemide in Water for Injections prepared with the aid of Sodium Hydroxide.

Frusemide Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of frusemide, $C_{12}H_{11}ClN_2O_5S$.

Usual strength. 10 mg per ml.

Description. A clear, colourless or almost colourless solution.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows three absorption maxima at about 228 nm, 271 nm and 333 nm.

B. To a volume of the injection containing 5 mg of Frusemide add 10 ml of *dilute hydrochloric acid* and boil under a reflux condenser on a water-bath for 15 minutes. Cool, add 15 ml of *1 M sodium hydroxide* and 5 ml of a 0.1 per cent w/v solution of *sodium nitrite*. Allow to stand for 3 minutes, add 5 ml of a 0.5 per cent w/v solution of *ammonium sulphamate*, mix and add 5 ml of a 0.1 per cent w/v solution of *N- (1-naphthyl) ethylenediamine dihydrochloride*; a red-violet colour is produced.

Tests

pH (2.4.24). 8.0 to 9.3.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dilute a volume of injection containing about 0.1 g of Frusemide to 100.0 ml with the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as ChromSpher C8),
- mobile phase: mixture of 70 volumes of solution prepared by dissolving 0.2 g of *potassium dihydrogen phosphate* and 0.25 g of *cetrimide* in 70 ml of *water* adjusted to pH 7.0 with *ammonia* and 30 volumes of *propanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 100 µl.

Inject the reference solution and the test solution. Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak obtained with the reference solution (1 per cent), and sum of areas of all the secondary peaks is not more than 1.5 times the area of the peak in the chromatogram obtained with the reference solution (1.5 per cent).

Bacterial endotoxins (2.2.3). Not more than 3.5 Endotoxin Units per mg of frusemide.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute a measured volume of the injection containing about 20 mg of Frusemide with *water* to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_{12}H_{11}ClN_2O_5S$ taking 580 as the specific absorbance at 271 nm.

Storage. Store protected from light.

Frusemide Tablets

Furosemide Tablets

Frusemide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of frusemide, $C_{12}H_{11}ClN_2O_5S$.

Usual strengths. 20 mg; 40 mg.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows three absorption maxima at about 228 nm, 271 nm and 333 nm.

B. Shake a quantity of the powdered tablets containing 80 mg of Frusemide with 10 ml of *ethanol (95 per cent)*, filter and evaporate the filtrate to dryness. Dissolve 25 mg of the residue obtained in 2.5 ml of *ethanol (95 per cent)* and add 2 ml of *4-dimethylaminobenzaldehyde reagent*; a green colour is produced which changes to deep red.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 5.8*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 277 nm (2.4.7). Calculate the content of $C_{12}H_{11}ClN_2O_5S$, in the medium from the absorbance obtained by using a solution of known concentration of *furosemide IPRS*.

Q. Not less than 70 per cent of the stated amount of $C_{12}H_{11}ClN_2O_5S$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Disperse a quantity of powdered tablets containing about 20 mg of Frusemide in 50.0 ml of the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as ChromSpher C8),
- mobile phase: a mixture of 70 volumes of solution prepared by dissolving 0.2 g of *potassium dihydrogen phosphate* and 0.25 g of *cetrimide* in 70 ml of *water* adjusted to pH 7.0 with *ammonia* and 30 volumes of *propanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 100 µl.

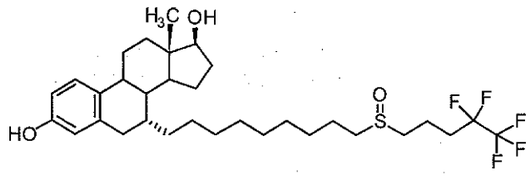
Inject the reference solution and the test solution. Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak obtained with the reference solution (1 per cent), and sum of areas of all the secondary peaks is not more than 1.5 times the area of the peak in the chromatogram obtained with the reference solution (1.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of Frusemide and shake with 150 ml of *0.1 M sodium hydroxide* for 10 minutes. Add sufficient *0.1 M sodium hydroxide* to produce 250.0 ml and filter. Dilute 5.0 ml to 200.0 ml with *0.1 M sodium hydroxide* and measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_{12}H_{11}ClN_2O_5S$ taking 580 as the specific absorbance at 271 nm.

Storage. Store protected from light.

Fulvestrant



$C_{32}H_{47}F_5O_3S$ Mol. Wt. 607.0

Fulvestrant is 7α-[9-[(*RS*)-(4,4,5,5,5-pentafluoropentyl) sulfinyl]nonyl]estra-1,3,5(10)-triene-3,17β-diol.

Fulvestrant contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{32}H_{47}F_5O_3S$, calculated on the anhydrous basis.

Category. Oestrogen receptor antagonist.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fulvestrant IPRS* or with the reference spectrum of fulvestrant.

B. Enantiomeric purity (See Test).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1).

Specific optical rotation (2.4.22). + 108.0° to + 115.0°, determined at 365 nm in a 2.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *methanol* and dilute to 5.0 ml with *methanol*.

Reference solution (a). A 1.0 per cent w/v solution of *fulvestrant IPRS* in *methanol*.

Reference solution (b). Dilute 1.0 ml of the reference solution (a) to 100.0 ml with *methanol*. Dilute 1.0 ml of the solution to 10.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octaylsilane bonded to porous silica (3.5 µm),

- column temperature: 40°,
- mobile phase: A. a mixture of 27 volumes of *methanol*, 32 volumes of *acetonitrile* and 41 volumes of *water*,
  B. a mixture of 10 volumes of *water*, 41 volumes of *methanol* and 49 volumes of *acetonitrile*,
- a gradient program using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
25	100	0
55	0	100
65	0	100
70	100	0

Name	Relative retention time	Correction factor
Fulvestrant impurity F[1]	0.4	0.3
Fulvestrant (Retention time: about 23 minutes)	1.0	—
Fulvestrant impurity A[2]	1.1	—
Fulvestrant impurity B[3]	1.2	—
Fulvestrant impurity C[4]	1.7	—
Fulvestrant impurity D[5]	1.9	0.7

[1]7-[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]-3,17â-dihydroxyestra-1,3,5(10)-trien-6-one (6-keto-fulvestrant).

[2]7β-[9-[(*RS*)-(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]estra-1,3,5(10)-triene-3,17β-diol(7β-fulvestrant),

[3]7α-[9-[(4,4,5,5,5-pentafluoropentyl)sulfonyl]nonyl]estra-1,3,5(10)-triene-3,17β - diol,

[4]7-[9-[[9-[(4,4,5,5,5-pentafluoropentyl)sulfinyl]nonyl]sulfinyl]nonyl] estra-1,3,5(10)-triene-3,17β-diol,

[5]7'-nonane-1,9-diylbis[estra-1,3,5(10)-triene-3,17β -diol].

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to fulvestrant impurity D is not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent.), the area of any peak corresponding to fulvestrant impurity C is not more than 3 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any peak corresponding to fulvestrant impurity B is not more than twice the area of principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any peak corresponding to fulvestrant impurity F is not more than 1.5 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), Ignore any peak with an area less than 0.5 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution. Dissolve 5 mg of *fulvestrant IPRS* in the mobile phase and dilute to 5.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica gel AD for chiral separation (10 µm),
- mobile phase: a mixture of 12 volumes of *ethanol* and 88 volumes of *2-methylpentane*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

The relative retention time with reference to fulvestrant epimer B (retention time: about 26 minutes) for fulvestrant epimer A is about 1.1.

Inject the reference solution. The test is not valid unless the resolution between the fulvestrantepimer B and fulvestrant epimer A is not less than 1.3.

Inject the reference solution and the test solution. Run the chromatogram 1.75 times the retention time of fulvestrantepimer B. Fulvestrantepimer A/ fulvestrantepimer B ratio: 42:58 to 48:52.

Heavy metals (2.3.13). Dissolve 1.0 g of substance under examination in 20.0 ml of *ethanol (95 per cent)*. 12 ml of the solution complies with the limit test for heavy metals, Method D (20 ppm), using 10 ml of *lead standard solution (1 ppm Pb)*.

Water (2.3.43). Not more than 0.5 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined in a platinum crucible.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{32}H_{47}F_5O_3S$.

Fulvestrant intended for use in the manufacture of parenteral preparations without further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirements.

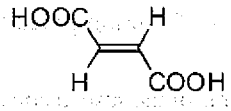
Bacterial endotoxins (2.2.3). Not more than 1.25 Endotoxin Unit per mg of fulvestrant.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture at a temperature between 2° to 8°. If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Fumaric Acid



$C_4H_4O_4$ Mol. Wt. 116.1

Fumaric acid is (2*E*)-butanedioic acid.

Fumaric Acid contains not less than 99.5 per cent and not more than 100.5 per cent of $C_4H_4O_4$, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Description. A white granules or crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fumaric acid IPRS* or with the reference spectrum of fumaric acid.

Tests

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1 g.

Maleic acid. Not more than 0.1 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.0001 per cent w/v solution of *maleic acid IPRS* in the mobile phase.

Reference solution (b). A 0.001 per cent w/v solution of *fumaric acid IPRS* and 0.0005 per cent w/v solution of *maleic acid IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 22 cm x 4.6 mm, strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 to 11µm),
- mobile phase. *0.0025 M sulphuric acid,*
- flow rate: 0.3 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 5 µl.

Inject reference solution (b). The test is not valid unless the resolution obtained with reference solution (b), between the peaks corresponding to maleic acid and fumaric acid is not less than 2.5 and relative standard deviation for replicate injections obtained with reference solution (a) is not more than 2.0 per cent. The relative retention time with reference to fumaric acid for maleic acid is about 0.5.

Inject reference solution (a) and the test solution.

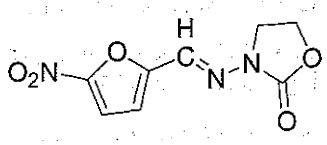
Calculate the content of maleic acid, $C_4H_4O_4$.

Assay. Weigh 1 g of Fumaric Acid and dissolve in 50 ml of *methanol,* warm gently on a steam bath to effect solution, cool. Titrate with *0.5 M sodium hydroxide*, using *phenolphthalein solution* as indicator. Carry out a blank titration.

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.02902 g of $C_4H_4O_4$.

Storage. Store protected from moisture.

Furazolidone



$C_8H_7N_3O_5$ Mol. Wt. 225.2

Furazolidone is 3-(5-nitrofurfurylideneamino)oxazolidin-2-one.

Furazolidone contains not less than 97.0 per cent and not more than 103.0 per cent of $C_8H_7N_3O_5$, calculated on the dried basis.

Category. Antibacterial; antiprotozoal.

Description. A yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *furazolidone IPRS* or with the reference spectrum of furazolidone.

B. Dissolve 1 mg in 1 ml of *dimethylformamide* and add 0.05 ml of *1 M ethanolic potassium hydroxide*; a deep blue colour is produced.

Tests

pH (2.4.24). 4.5 to 7.0, determined in a solution prepared by shaking 1.0 g for 15 minutes with 100 ml of *carbon dioxide-free water* and filtering.

Nitrofurfural diacetate. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 95 volumes of *toluene* and 5 volumes of *dioxan*.

Test solution. Dissolve 50 mg of the substance under examination in 5 ml of *dimethylformamide* by heating on a water-bath for a few minutes, allow to cool and dilute to 10 ml with *acetone*.

Reference solution. A solution containing 0.01 per cent w/v of *nitrofurfural diacetate IPRS* in a mixture of equal volumes of *dimethylformamide* and *acetone*.

Apply to the plate 10 µl of the reference solution and 20 µl of the test solution. After development, dry the plate in air and heat it at 105° for 5 minutes. Spray with a solution prepared by dissolving 0.75 g of *phenylhydrazine hydrochloride* in 10 ml of *ethanol (95 per cent)*, diluting to 50 ml with *water*, adding *activated charcoal*, filtering and then adding 25 ml of *hydrochloric acid* and sufficient *water* to produce 200 ml. Any spot corresponding to nitrofurfural diacetate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. *Carry out the following procedure protected from light.*

Weigh 80 mg, add 150 ml of *dimethylformamide*, swirl to dissolve and add sufficient *water* to produce 500.0 ml. Dilute 5.0 ml to 100.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7). Calculate the content of $C_8H_7N_3O_5$ taking 750 as the specific absorbance at 367 nm.

Storage. Store protected from light.

Furazolidone Oral Suspension

Furazolidone Oral Suspension is a suspension of Furazolidone in a suitable aqueous flavoured vehicle.

Furazolidone Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of furazolidone, $C_8H_7N_3O_5$.

Usual strength. 25 mg per 5 ml.

Identification

Add a quantity of the suspension containing 50 mg of Furazolidone to 10 ml of a freshly prepared mixture of 9 volumes of *dimethylformamide* and 1 volume of *1 M ethanolic potassium hydroxide*. The solution turns purple, immediately changes to deep blue and on standing for about 10 minutes, again turns purple.

Tests

pH (2.4.24). 6.0 to 8.5.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. *Carry out the following procedure protected from light.*

To a measured volume of the suspension containing 50 mg of Furazolidone add 5 ml of *water* and mix. Transfer this mixture to a 250-ml volumetric flask with the aid of *dimethylformamide*. Add about 150 ml of *dimethylformamide*, shake by mechanical means for 10 minutes, dilute to volume with *dimethylformamide* and mix. Dilute 5.0 ml of the solution to 100.0 ml with *water* and mix well. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7), using *dimethylformamide solution (5 per cent v/v)* as the blank. Calculate the content of $C_8H_7N_3O_5$ taking 750 as the specific absorbance at 367 nm.

Storage. Store protected from light at a temperature not exceeding 30°.

Furazolidone Tablets

Furazolidone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of furazolidone, $C_8H_7N_3O_5$.

Usual strengths. 100 mg; 200 mg.

Identification

To a quantity of the powdered tablets containing 50 mg of Furazolidone add 10 ml of a freshly prepared mixture of 9 volumes of *dimethylformamide* and 1 volume of *1 M ethanolic potassium hydroxide*. The solution turns purple,

immediately changes to deep blue and on standing for 10 minutes, again turns purple.

Tests

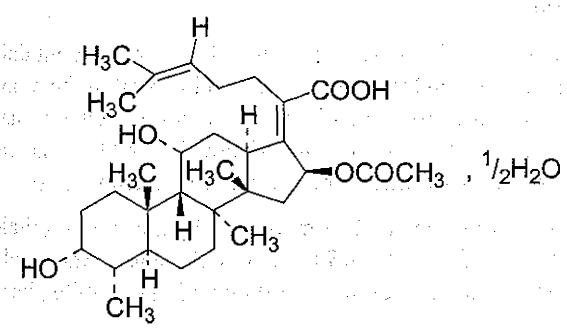
Other tests. Comply with the tests stated under Tablets.

Assay. *Carry out the following procedure protected from light.*

Weigh and powder 20 tablets. Disperse a quantity of the powder containing 80 mg of Furazolidone into a 200-ml volumetric flask, add 100 ml of *dimethylformamide*, warm to about 50° and shake well. Cool, dilute to volume with *dimethylformamide*, mix and centrifuge a small quantity of the mixture. Dilute 5.0 ml of the clear, supernatant liquid to 250.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7), using *dimethylformamide* diluted 50 times with *water* as the blank. Calculate the content of $C_8H_7N_3O_5$ taking 750 as the specific absorbance at 367 nm.

Storage. Store protected from light at a temperature not exceeding 30°.

Fusidic Acid



$C_{31}H_{48}O_6,½H_2O$ Mol. Wt. 525.7

Fusidic Acid is *ent*-16α-acetyloxy-3β,11β-dihydroxy-4β,8β,14β-trimethyl-18-nor-5β,10α-cholesta(17*Z*)-17(20),24-dien-21-oic acid hemihydrate, an antimicrobial substance produced by the growth of certain strains of *Fusidium coccineum* or by any other means.

Fusidic Acid contains not less than 97.5 per cent and not more than 101.0 per cent of $C_{31}H_{48}O_6$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *fusidic acid IPRS* or with the reference spectrum of fusidic acid.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G254*.

Mobile phase. A mixture of 80 volumes of *chloroform*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 2.5 volumes of *methanol*.

Test solution. Dissolve 0.2 g of the substance under examination in 100.0 ml of *ethanol (95 per cent)*.

Reference solution. A 0.24 per cent w/v solution of *diethanolamine fusidate IPRS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 105° for 10 minutes and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.17).

Test solution. Dissolve 0.5 g of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dissolve 5 mg of *3-ketofusidic acid IPRS* in 5 ml of the mobile phase. To 1.0 ml of the solution add 0.2 ml of the test solution and dilute to 20.0 ml with the mobile phase.

Reference solution (b). Dilute 20 µl of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 20 volumes of *water*, 20 volumes of a 1 per cent w/v solution of *phosphoric acid* and 10 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Continue the chromatography for at least 3.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than 4 times the area of the peak corresponding to fusidic acid in the chromatogram obtained with reference solution (a). Ignore any peak with an area less than that of the principal peak in the chromatogram obtained with reference

solution (b). The test is not valid unless the resolution factor between the peaks corresponding to 3-ketofusidic acid and fusidic acid in the chromatogram obtained with reference solution (a) is not less than 2.5 and unless the principal peak in the chromatogram obtained with reference solution (b) has a signal-to-noise ratio of not less than 3.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). 1.4 to 2.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 250 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.05 per cent w/v solution of *fusidic acid IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 20 volumes of *water*, 20 volumes of a 1.0 per cent w/v solution of *orthophosphoric acid* and 10 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

The retention time of the principal peak is about 6.0 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{31}H_{48}O_6$.

Storage. Store protected from light.

Fusidic Acid Cream

Fusidic Acid Cream contains fusidic acid in a suitable cream base.

Fusidic Acid Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of fusidic acid, $C_{31}H_{48}O_6$.

Usual strength. 2 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2.5 volumes of *methanol*, 10 volumes of *glacial acetic acid*, 10 volumes of *cyclohexane* and 80 volumes of *chloroform*.

Test solution. Disperse a quantity of the cream with shaking containing about 40 mg of anhydrous fusidic acid in 10 ml of *ethanol (95 per cent)*, filter. Use the filtrate.

Reference solution (a). A 0.5 per cent w/v solution of *diethanolamine fusidate IPRS* in *ethanol (95 per cent)*.

Reference solution (b). A 0.05 per cent w/v solution of *potassium sorbate* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12.5 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a) and is well separated from any spot corresponding to the spot in the chromatogram obtained with reference solution (b).

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 6.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the cream containing about 15 mg of anhydrous fusidic acid in 25 ml of the mobile phase, heat the mixture until the cream has melted and shake for 15 minutes. Cool the mixture to below 10° and filter. Discard the first few ml of filtrate and warm to room temperature.

Reference solution (a). To 1 ml of a 0.06 per cent w/v solution of *3-ketofusidic acid IPRS* in the mobile phase. Add 1 ml of the test solution and dilute to 100 ml with the mobile phase.

Reference solution (b). Dilute 30 µl of the test solution to 100.0 ml with the mobile phase.

Reference solution (c). A 0.004 per cent w/v solution of *potassium sorbate* in the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Lichrospher 100 RP-18),
- mobile phase: a mixture of 10 volumes of *methanol*, 40 volumes of *0.05M orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to 3-ketofusidic acid and fusidic acid is not less than 3.5.

Inject reference solution (a), (b), (c) and the test solution. Run the chromatogram 3.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent) and any peak with the same retention time as that of the principal peak in the chromatogram obtained with reference solution (c).

Other tests. Comply with the tests stated under Cream.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the cream containing about 15 mg of anhydrous fusidic acid in 50 ml of the mobile phase, heat until the cream has melted and shake for 15 minutes. Cool the mixture to below 10° and filter. Discarding the first few ml of filtrate and warm to room temperature.

Reference solution. A 0.0375 per cent w/v solution of *diethanolamine fusidate IPRS* in the mobile phase.

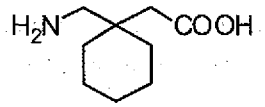
Chromatographic system as described under Related substances.

Inject the reference solution and the test solution.

Calculate the content of $C_{31}H_{48}O_6$ in the cream.

G

Gabapentin



$C_9H_{17}NO_2$ Mol. Wt. 171.2

Gabapentin is [1-(aminomethyl) cyclohexyl] acetic acid

Gabapentin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_9H_{17}NO_2$, calculated on the anhydrous basis.

Category. *Antiepileptic.*

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gabapentin IPRS* or with the reference spectrum of gabapentin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 6.5 to 8.0, determined in a 2.0 per cent solution.

Related substances. A. Determine by liquid chromatography (2.4.14).

Diluent. Dissolve 2.32 g of *ammonium dihydrogen phosphate* in 950 ml of *water*, and adjusted to pH 2.0 with *orthophosphoric acid*, and dilute to 1000 ml with *water*.

Test solution. Dissolve 140 mg of the substance under examination in the diluent and dilute to 10.0 ml with the diluent.

Reference solution (a). A solution containing 1.4 per cent of *gabapentin IPRS*, 0.0014 per cent *gabapentin impurity A IPRS* and 0.00084 per cent *gabapentin impurity B IPRS* in the diluent.

Reference solution (b). A 0.00084 per cent w/v solution of *gabapentin impurity E IPRS* in the diluent.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 24 volumes of *acetonitrile* and 76 volumes of buffer solution prepared by dissolving 0.58 g of *ammonium dihydrogen phosphate* and 1.83 g of *sodium perchlorate* in 950 ml of *water* and adjusted to pH 1.8 with *perchloric acid* and diluted to 1000 ml with *water*,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Gabapentin	1.0	---
Gabapentin impurity E[1]	2.9	—
Gabapentin impurity A[2]	3.5	0.188
Gabapentin impurity B[3]	3.8	2.86

[1]carboxymethyl-cyclohexanecarboxylic acid,

[2]2-aza-spiro [4.5] decan-3-one,

[3](1-cyano-cyclohexyl)-acetic acid.

NOTE—The relative response factor are calculated in response of gabapentin impurity E due to low absorptivity of gabapentin at 254 nm.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to gabapentin impurity A and gabapentin impurity B is not less than 2.3.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to gabapentin impurity E is not more than 1.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent),the area of peak corresponding to gabapentin impurity A is not more than 1.6 times the area of principal peak obtained with reference solution (b) (0.1 per cent) and the area of peak corresponding to gabapentin impurity B is not more than the area of principal peak obtained with reference solution (b) (0.06 per cent), the area of any other secondary peak multiplied with a correction factor of 2.43 is not more than 1.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

B. Determine by liquid chromatography (2.4.14) as described in test A of related substances with the following modifications.

Reference solution. A 0.00028 per cent w/v solution of *gabapentin impurity D IPRS (1-(3-oxo-2-aza-spiro[4.5]dec-2-ylmethyl)-cyclohexyl)-acetic acid* in the diluent.

Use chromatographic systems as described in related substances test A with the following modifications.

- mobile phase: a mixture of 30 volumes of *methanol*, 35 volumes of *acetonitrile* and 35 volumes of buffer solution prepared by dissolving 0.58 g of *ammonium dihydrogen phosphate* and 1.83 g of *sodium perchlorate* in 950 ml of *water* and adjusted to pH 1.8 with *perchloric acid* and dilute to 1000 ml with *water*.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 13,600 theoretical plates.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak other than impurity D is not more than 5 times the area of principal peak obtained with the reference solution (0.1 per cent).

The total impurity for test A and B is not more than 0.5 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 0.5 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14), as described under the test A of Related substances with the following modifications.

Reference solution (a). A 1.4 per cent w/v solution of *gabapentin IPRS* in the diluent.

Reference solution (b). A 0.23 per cent w/v solution of *gabapentin IPRS* from reference solution (a) in the diluent.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 1900 theoretical plates.

Inject reference solution (a) and the test solution.

Calculate the content of $C_9H_{17}NO_2$.

Storage. Store protected from moisture.

Gabapentin Capsules

Gabapentin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of gabapentin $C_9H_{17}NO_2$.

Usual strengths. 100 mg; 300 mg; 400 mg; 450 mg.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gabapentin IPRS* or with the reference spectrum of gabapentin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.06 M hydrochloric acid*,
Speed and time. 50 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *gabapentin IPRS* in the dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 940 ml of the buffer solution prepared by dissolving 1.2 g of *potassium dihydrogen orthophosphate* adjusted to pH 6.9 with *5 M potassium hydroxide* and 60 ml of *acetonitrile*,
  B. a mixture of 700 ml of the buffer solution prepared by dissolving 1.2 g of *potassium dihydrogen orthophosphate* adjusted to pH 6.9 with *5 M potassium hydroxide* and 300 ml of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
4	100	0
45	0	100
45.1	100	0
50	100	0

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 for the principal peak.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to gabapentin impurity A is not more than twice the area of peak due to gabapentin impurity A in the chromatogram obtained with the reference solution (0.4 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent), and the sum of the area of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Diluent. Dissolve 1.2 g of *potassium dihydrogen orthophosphate* in 1000 ml *water* and adjusted to pH 6.9 with *5 M potassium hydroxide*.

Test solution. Weigh a quantity of mixed content of 20 capsules. Disperse a quantity of powder containing 400 mg gabapentin in the diluent, mix with the aid of ultrasound for 60 seconds and dilute to 100.0 ml with the diluent.

Reference solution. A 0.4 per cent w/v solution of *gabapentin IPRS* in the diluent.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 940 ml of buffer prepared by dissolving 1.2 g of *potassium dihydrogen orthophosphate* in *water* adjusted to pH 6.9 using *5 M potassium hydroxide* and 60 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 7000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_{17}NO_2$ in the capsule.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Gabapentin Tablets

Gabapentin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of gabapentin, $C_9H_{17}NO_2$.

Usual strengths. 100 mg; 300 mg; 400 mg; 600 mg; 800 mg.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gabapentin IPRS* or with the reference spectrum of gabapentin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.06 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, diluted if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *gabapentin IPRS* in dissolution medium to obtain a solution of known concentration similar to the test solution.

Use the chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_{17}NO_2$.

Q. Not less than 80 per cent of the stated amount of $C_9H_{17}NO_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Diluent. Dissolve 1.2 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and adjusted to pH 6.9 with *5 M potassium hydroxide*.

Test solution. Weigh and powder 20 tablets. Dissolve a weighed quantity containing about 500 mg gabapentin in diluent, sonicate for 30 minutes, dilute to 25.0 ml with the diluent.

Reference solution. A solution containing 0.004 per cent w/v each of *gabapentin IPRS* and *gabapentin impurity A IPRS (2-Aza-spiro[4, 5] decan-3-one)* in the diluent.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 940 ml of buffer prepared by dissolving 1.2 g of *potassium dihydrogen orthophosphate* in *water* adjusted to pH 6.9 using *5 M potassium hydroxide* and 60 volumes of *acetonitrile*,
  B. a mixture of 700 ml of buffer prepared by dissolving 1.2 g of *potassium dihydrogen orthophosphate* in *water* adjusted to pH 6.9 using *5 M potassium hydroxide* and 300 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
4	100	0
45	0	100
45.1	100	0
50	100	0

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 for the principal peak.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to gabapentin impurity A is not more than twice the area of peak due to gabapentin impurity A in the chromatogram obtained with the reference solution (0.4 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent), and the sum of the area of all the secondary peak in not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Diluent. Dissolve 1.2 g of *potassium dihydrogen orthophosphate* in 1000 ml *water* and adjusted to pH 6.9 with *5 M potassium hydroxide*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 400 mg gabapentin in the diluent, sonicate for 30 minutes, dilute to 100.0 ml with diluent.

Reference solution. A 0.4 per cent w/v solution of *gabapentin IPRS* in the buffer solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 940 volumes of buffer solution prepared by dissolving 1.2 g of *potassium dihydrogen orthophosphate* in *water* adjusted to pH 6.9 using *5 M potassium hydroxide* and 60 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

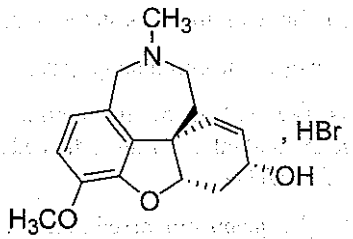
Inject the reference solution. The test is not valid unless the column efficiency is not less than 7000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_{17}NO_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Galantamine Hydrobromide



$C_{17}H_{21}NO_3,HBr$ Mol Wt. 368.3

Galantamine Hydrobromide is (4a*S*,6*R*,8a*S*)- 5,6,9,10,11,12-Hexahydro- 3-methoxy- 11-methyl- 4a*H*- [1]benzofuro[3a,3,2-*ef*] [2] benzazepin- 6-ol hydrobromide.

Galantamine Hydrobromide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{21}NO_3,HBr$, calculated on the dried basis.

Category. Cholinesterase Inhibtor.

Description. A white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *galantamine hydrobromide IPRS* or with the reference spectrum of galantamine hydrobromide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Galantamine Enantiomer. Not more than 0.1 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of substance under examination in *methanol* and dilute to 100.0 ml with *methanol*.

Reference solution. A solution containing 0.0005 per cent w/v each of (+) *galantamine IPRS* and *galantamine hydrobromide IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, such as chiral Pack-AD-H, (5 µm),
- mobile phase: a mixture of 85 volumes of *n-hexane*, 15 volumes of *isopropylalcohol*, 0.1 volume of *diethylamine* and 0.1 volume of *trifluoroacetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peak due to galantamine and (+) galantamine isomer is not less than 3.0.

Inject the reference solution and the test solution.

Calculate the content of Galantamine isomer by area normalisation.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 95 volumes of *water* and 5 volumes of *methanol*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.1 per cent w/v each of *galantamine IPRS*, *galantamine N-oxide IPRS*, *lycoramine IPRS*, *epigalantamine IPRS and dehydroxyl galantamine IPRS* in the solvent mixture.

Reference solution (b). A 0.0005 per cent w/v solution of *galantamine hydrobromide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 55°,
- mobile phase: A. a mixture of 95 volumes of a solution prepared by dissolving 0.79 g of *disodium hydrogen orthophosphate* and 2.46 g of *sodium dihydrogen orthophosphate anhydrous* into 1000 ml of *water* and 5 volumes of *methanol*.

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
6	100	0
20	95	05
35	85	15
50	80	20
51	40	60
55	40	60
56	100	0
65	100	0

The relative retention time with reference to galantamine for norgalantamine is 0.27; for desmethyl galantamine is 0.35, for galantamine-N-oxide is 0.62; for lycoramine is 0.82; for epigalantamine is 1.17 and for dehydroxyl galantamine is 2.07.

Inject reference solution (a) and (b). The test is not valid unless the resolution between galantamine and epigalantamine is not less than 4.5 in the chromatogram obtained with reference solution (a) in the chromatogram obtained with reference solution (b), the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any peak due to, galantamine-N-oxide, epigalantamine, desmethyl galantamine is not more than 0.2 per cent, the area of any peak due to de hydroxyl galantamine and lycoramine, is not more than 0.4 per cent , the area of any other secondary peak is not more than 0.1 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalisation.

Bromide content. 20.6 per cent to 22.8 per cent.

Dissolve 0.35 g in 40.0 ml of *water*, add 10.0 ml of *nitric acid*. Titrate with *0.1 M silver nitrate*, determining the end point potentiometrically (2.4.25), using combined electrode. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.00799 g of bromide.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water* and 40 volumes of *methanol*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with

the solvent mixture and further dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *galantamine hydrobromide IPRS* in the solvent mixture.

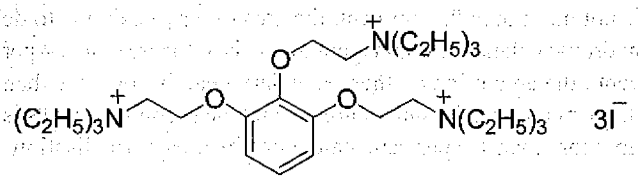
Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 90 volumes of a solution prepared by dissolving 2.64 g of *diammonium hydrogen orthophosphate* to 1000 ml of *water*, adjusted to pH 6.8 with *orthophosphoric acid* and 10 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{21}NO_3$,HBr.

Gallamine Triethiodide



$C_{30}H_{60}I_3N_3O_3$ Mol. Wt. 891.5

Gallamine Triethiodide is 2,2',2"-(benzene-1,2,3-triyltrioxy)tris(tetraethylammonium) triiodide.

Gallamine Triethiodide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{30}H_{60}I_3N_3O_3$, calculated on the dried basis.

Category. Skeletal muscle relaxant.

Description. A white or almost white powder; hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gallamine triethiodide IPRS*.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum at about 225 nm is 0.50 to 0.55.

C. To 5 ml of a 1 per cent w/v solution add 1 ml of *potassium mercuri-iodide solution*; a yellow precipitate is produced.

D. Acidify 2 ml of a 0.5 per cent w/v solution with 0.2 ml of *2 M nitric acid*; the resulting solution gives reaction (A) of iodides (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution is clear (2.4.1), and, when examined immediately after preparation, not more intensely coloured than reference solution YS7 (2.4.1).

Acidity or alkalinity. To 50 ml of *water* add 0.2 ml of *methyl red solution* and adjusted to pH 6.0 with *0.01 M sulphuric acid* or *0.02 M sodium hydroxide* until the colour is orange-yellow. Add 1 g of the substance under examination and shake to dissolve. Not more than 0.2 ml of *0.01 M sulphuric acid* or *0.02 M sodium hydroxide* is required to restore the orange-yellow colour.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 1.4 g of *sodium perchlorate* in 85 ml of *phosphate buffer pH 3.0* and 15 ml of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

The relative retention time with reference to gallamine for 2,2',2"-[benzene-1,2,3-triyltris(oxy)]tris(*N,N*-diethylamine) (gallamine impurity A) is about 0.45, for 2,2'-[2-[2-(triethylammonio)ethyl]-1,3-phenylenebis(oxy)]bis(*N,N,N*-triethylethanaminium)triiodide (gallamine impurity B) is about 0.5, for 2,2'-[2-[2-(diethylmethylammonio)ethoxy]-1,3-phenylene bis(oxy)]bis(*N,N,N*-triethylethanaminium)triiodide (gallamine impurity C) is about 0.65, for 2,2'-[3-[2-(diethylmethylammonio)ethoxy]-1,2-phenylenebis(oxy)bis (*N,N,N*-triethylethanaminium)triiodide (gallamine impurity D) is about 0.75, for 2,2'-[3-[2-(diethylamino)ethoxy-1,2-phenylenebis (oxy)bis(*N,N,N*-triethylethanaminium)diiodide (gallamine impurity E) is about 0.85, for 2,2',2"-[4-[2-(triethylammonio) ethyl]benzene-1,2,3triyltris(oxy)tris(*N,N,N*-triethyl-ethanaminium)tetraidide (gallamine impurity F) is about 0.9.

Inject the reference solution and the test solution. Run the chromatogram 1.5 times the peak due to triethylgallamine as perchlorate. In the chromatogram obtained with the test solution the area of secondary peak corresponding to gallamine impurity A, B, C, D, E and F is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore the peak due to iodide (non-retained peak).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.27 g and dissolve in a mixture of 40 ml of *acetone* and 15 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02972 g of $C_{30}H_{60}I_3N_3O_3$.

Storage. Store protected from light.

Gallamine Injection

Gallamine Triethiodide Injection

Gallamine Injection is a sterile solution of Gallamine Triethiodide in Water for Injections.

Gallamine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of gallamine triethiodide, $C_{30}H_{60}I_3N_3O_3$.

Usual strength. 40 mg per ml.

Description. A clear, colourless or almost colourless solution.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 225 nm.

B. To 1 ml add 1 ml of iodinated *potassium iodide solution*; a brown precipitate is produced.

C. To 1 ml add 1 ml of *potassium mercuri-iodide solution*; a yellow precipitate is produced.

Tests

pH (2.4.24). 5.5 to 7.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing 60 mg of Gallamine Triethiodide in 100.0 ml of the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica whose surface is chemically modified with a mixture of chemically- bonded octylsilane and octadecylsilane groups (5µm) (Such as Hichrom RPB),
- column temperature: 40°,
- mobile phase: dissolve 14 g of *sodium perchlorate* in 850 ml of a solution prepared by diluting 0.7 ml of *orthophosphoric acid* to 900 ml with *water*, adjusted to pH 3.0 with *10 M sodium hydroxide* and dilute to 1000 ml with *water*, and add 130 ml of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak obtained with the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principle peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore the peak due to iodide (non-retained peak).

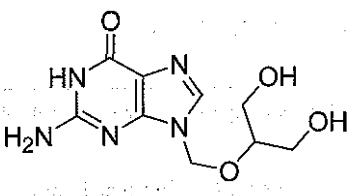
Bacterial endotoxins (2.2.3). Not more than 5.0 Endotoxin Units per mg of gallamine triethiodide.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute a measured volume containing 40 mg of Gallamine Triethiodide with sufficient *0.01 M hydrochloric acid* to produce 200.0 ml. Dilute 5.0 ml of the solution to 100.0 ml with *0.01 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 225 nm (2.4.7). Calculate the content of $C_{30}H_{60}I_3N_3O_3$ taking 525 as the specific absorbance at 225 nm.

Storage. Store protected from light.

Ganciclovir



$C_9H_{13}N_5O_4$ Mol Wt. 255.2

Ganciclovir is 2-amino-9-[[2-hydroxy-1-(hydroxymethyl) ethoxy]methyl]-1,9-dihydro-6H-purin-6-one.

Ganciclovir contains not less than 98.0 per cent and not more than 102.0 per cent of $C_9H_{13}N_5O_4$, calculated on the anhydrous basis.

Category. Antiviral.

Description. A white or almost white, powder; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ganciclovir IPRS* or with the reference spectrum of ganciclovir.

B. When examined in the range 210 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 254 nm.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 11 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.01 per cent w/v each of *ganciclovir IPRS* and *ganciclovir related compound A IPRS* in the mobile phase.

Reference solution (b).A 0.022 per cent w/v solution of *ganciclovir IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm in diameter,
- column temperature: 40°,
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of a solution prepared by dissolving 0.5 ml of *trifluoroacetic acid* in 1000 ml of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Name	Relative retention time
Ganciclovir related compound A[1]	0.9
Ganciclovir	1.0

[1] 2-Amino-9-[(2,3-dihydroxypropoxy)methyl]-1,9-dihydro-6*H*-purin-6-one (isoganciclovir).

Inject reference solution (a).The test is not valid unless the resolution between the ganciclovir related compound A and ganciclovir is not less than 1.4, the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the test solution. The area of any peak corresponding to ganciclovir related compound A is not more than 0.5 per cent. The sum of areas of all secondary peaks is not more than 1.5 per cent, calculated by area normalization.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (a).The test is not valid unless the resolution between the ganciclovir related compound A and ganciclovir is not less than 1.4, the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 1.4.

Inject reference solution (b). The relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_9H_{13}N_5O_4$.

Storage. Store in well-closed containers, protected from moisture at a temperature below 30°.

Ganciclovir Injection

Ganciclovir Injection is a sterile freeze dried material consisting of Ganciclovir with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of sealed container in the requisite amount of sterile water for injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under parenteral preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ganciclovir Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ganciclovir, $C_9H_{13}N_5O_4$.

Usual strength. 500 mg per vial.

Description. A white to off-white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

pH (2.4.24). 10.8 to 11.4, determined on the constituted solution.

Water (2.3.43). Not more than 3.0 per cent, using a mixture of *anhydrous formamide* and *methanol* (1:1) instead of *methanol*.

Bacterial endotoxins. (2.2.3). Not more than 0.84 Endotoxin Unit per mg of ganciclovir.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. A 0.015 per cent w/v solution of *hypoxanthine* in *water*.

Test solution (a). Determine the weight of the contents of 10 containers. Transfer a weighed quantity of the mixed contents of the 10 containers containing 100 mg of Ganciclovir to a 100.0 ml volumetric flask, add about 80 ml of the *water* and dissolve by shaking and mixing if necessary, with the aid of ultrasound. Cool and dilute to 100.0 ml with the *water*.

Test solution (b). Dilute 5.0 ml of test solution (a) and add 10.0 ml internal standard solution and dilute to 100.0 ml with mobile phase.

Reference solution (a). A 0.025 per cent w/v solution of *ganciclovir IPRS* in *water*.

Reference solution (b). Dilute 20.0 ml of reference solution (a) and add 10.0 ml internal standard solution to 100.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: Dissolve 1.4 g of *monobasic ammonium phosphate* and 2.0 g of *phosphoric acid* in 1000 ml of *water* and mix,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (b). The relative retention times of hypoxanthine and ganciclovir are about 0.7 and 1.0 respectively. The test is not valid unless the resolution between the peaks due to hypoxanthine and ganciclovir is not less than 3.0, the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections in not more than 2.0 per cent.

Inject reference solution (b) and test solution (b).

Calculate the content of $C_9H_{13}N_5O_4$ in the injection.

Storage. Store protected from moisture, in a sterile tamper evident container, sealed so as to exclude micro organisms, at a temperature not exceeding 30°.

Labelling. Label state that it is to be handled with great care because it is a potent cytotoxic agent and suspected carcinogen. The label states the quantity of ganciclovir in the sealed container and procedure for reconstitution.

Ganciclovir Oral Suspension

Ganciclovir Oral Suspension is a suspension of Ganciclovir in a suitable vehicle.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Ganciclovir Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ganciclovir, $C_9H_{13}N_5O_4$.

The constituted suspension, when stored at the temperature and for the period stated on the label during which it may be expected to be satisfactory for use, contains not less than 80.0 per cent of the stated amount of ganciclovir, $C_9H_{13}N_5O_4$.

NOTE — Avoid skin contact or inhalation of ganciclovir by using protective gloves and a fume hood or surgical mask.

Usual strength. 100 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.0 to 5.0.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. A 0.04 per cent w/v solution of *hypoxanthine* in *water*.

Test solution (a). Mix the contents of not less than 2 constituted containers. Shake a quantity of oral suspension containing 100 mg of Ganciclovir with 100.0 ml with *water*. Dilute 10.0 ml of the solution to 100.0 ml with same solvent.

Test solution (b). Dilute 6.0 ml of test solution (a) and add 1.0 ml internal standard solution to 100.0 ml with *water*.

Reference solution. A solution containing 0.006 per cent w/v of *ganciclovir IPRS* in *water*. Mix 10.0 ml of the solution with 1.0 ml internal standard solution and dilute to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 2.5 volumes of *acetonitrile* and 97.5 volumes of *0.025M monobasic sodium phosphate* solution, adjusted to pH 2.5 with *orthophosphoric acid*, filter and degas,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

The relative retention time with respect to ganciclovir for hypoxanthine is 0.75.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the reference solution and test solution (b).

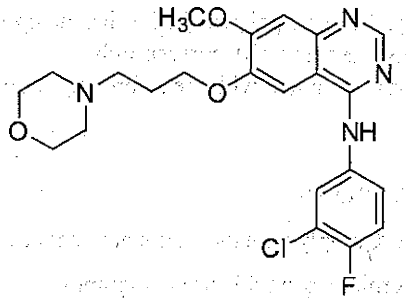
Calculate the content of $C_9H_{13}N_5O_4$ in the suspension.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_9H_{13}N_5O_4$, weight in volume.

Storage. Store protected from light and moisture.

Labelling. The constituted suspension should be used within 90 days.

Gefitinib



$C_{22}H_{24}O_3N_4FCl$ Mol. Wt. 446.9

Gefitinib is *N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(4-morpholinyl)propoxy]-4-quinazolinoamine.

Gefitinib contains not less than 98.0 per cent and not more than 102.0 per cent $C_{22}H_{24}O_3N_4FCl$, calculated on the dried basis.

Category. Anticancer.

Description. A white or off white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gefitinib IPRS* or with the reference spectrum of gefitinib.

Tests

Melting range (2.4.21). 193° to 197°.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in *methanol* and dilute to 100.0 ml with *methanol*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 40 volumes of 1 per cent w/v solution of *ammonium acetate* and 60 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak obtained in the chromatogram obtained with reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

Heavy metals (2.3.13). 2 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 100.0 ml of *methanol*.

Reference solution. A 0.01 per cent w/v solution of *gefitinib IPRS* in the *methanol*.

Chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}O_3N_4FCl$.

Storage. Store protected from light and moisture, at a temperature not exceeding 25°.

Gefitinib Tablets

Gefitinib Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of stated amount of gefitinib, $C_{22}H_{24}O_3N_4FCl$.

Usual strength. 250 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *acetate buffer pH 4.0*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Dilute a suitable volume of the filtrate with the same solvent, if necessary and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 254 nm. Calculate the content of $C_{22}H_{24}O_3N_4FCl$ in the medium from the absorbance obtained from a solution of known concentration of *gefitinib IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{24}O_3N_4FCl$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 100 mg of Gefitinib, disperse in 100 ml of the *methanol*, filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *gefitinib IPRS* in *methanol*. Dilute 5 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of 1.0 per cent w/v solution of *ammonium acetate* and 60 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{24}O_3N_4FCl$ in the tablets.

Storage. Store protected from light and moisture.

Gelatin

Gelatin is a purified protein obtained by partial hydrolysis of animal collagen. Gelatin used in the manufacture of capsule shells or as a pharmaceutical aid in the manufacture of tablets may contain suitable antimicrobial agents.

Category. Pharmaceutical aid (encapsulating agent; suspending agent; tablet binding and coating agent).

Description. Light amber to faintly yellow, translucent flakes, sheets, shreds, powder or granules; slight. Stable in air but is subject to microbial decomposition when moist or in solution.

Identification

A. Dissolve 1 g in sufficient *carbon dioxide-free water* at about 55° to produce 100 ml and maintain the solution at this temperature until required for use (solution A). To 2 ml of solution A add 0.05 ml of *copper sulphate solution*, mix and add 0.5 ml of *2 M sodium hydroxide*; a violet colour is produced.

B. Add 10 ml of *water* to 0.5 g in a test-tube, allow to stand for 10 minutes, heat at 60° for 15 minutes, allow to stand upright at 0° for 6 hours and invert the test-tube; the contents do not immediately flow out.

Tests

Appearance of solution. Solution A is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution YS4 (2.4.1).

pH (2.4.24). 3.8 to 7.6, determined in solution A.

Conductivity (2.4.9). Not more than 1 mS·cm^{-1}, determined on a 1.0 per cent solution at 30 ± 1.0°.

Iron. Not more than 30 ppm.

Determine by atomic absorption spectrometry (2.4.2), Method A at 248.3 nm.

Test solution. To 5.0 g of the substance under examination, in a conical flask, add 10 ml of *hydrochloric acid*. Close the flask and place in a water-bath at 75°-80° for 2 hours. Allow to cool and adjust the content of the flask with *water* to 100.0 g

Iron standard solution (8 ppm). Dissolve 80 mg of *iron* in 50 ml of 22.0 per cent w/v solution of *hydrochloric acid* and dilute to 1000.0 ml with *water*. Prepare a 1:10 dilution with *water*, immediately before use, dilute with *water* as necessary.

Chromium. Not more than 10 ppm.

Determine by atomic absorption spectrometry (2.4.2), Method A at 357.9 nm,

Test solution. As described under test for iron.

Chromium standard solution (100 ppm). A 0.0283 per cent w/v solution of dried *potassium dichromate* in *water*, dilute with *water* as necessary.

Zinc. Not more than 30 ppm.

Determine by atomic absorption spectrometry (2.4.2)., Method A at 213.9 nm

Test solution. As described under test for iron.

Zinc standard solution (10 ppm). Dissolve 0.440 g of zinc sulphate heptahydrate and 1 ml of a 30 per cent w/v solution of *acetic acid* in *water*, and dilute to 100.0 ml with *water*. Prepare a 1:100 dilution in *water*, immediately before use, dilute with *water* as necessary.

Sulphur dioxide. Not more than 50 ppm, determined by the following method.

Add 150 ml of *water* to a 500-ml three-necked, round-bottomed flask fitted with a water-cooled reflux condenser 200 mm long the upper end of which is connected to an absorption tube. The flask is fitted with a 100-ml dropping funnel and a gas inlet tube that reaches nearly to the bottom of the flask. Pass a stream of carbon dioxide through the flask at a rate of 100 ml per minute for 15 minutes. Connect an absorption tube containing 10 ml of *hydrogen peroxide solution (10 vol)* previously neutralised to a 0.1 per cent w/v solution of *bromophenol blue* in *ethanol (20 per cent)* and without interrupting the flow of carbon dioxide, introduce through the funnel 25 g of the substance under examination and 80 ml of *2 M hydrochloric acid*. Boil for 1 hour, disconnect the absorption tube and stop the flow of carbon dioxide. Wash the contents of the absorption tube into a 250-ml conical flask, heat on a water-bath for 15 minutes and allow to cool. Titrate with *0.01 M sodium hydroxide* using a 0.1 per cent w/v solution of *bromophenol blue* in *ethanol (20 per cent)* as indicator, until the colour changes from yellow to violet-blue.

1 ml of *0.01 M sodium hydroxide* is equivalent to 0.0003203 g of sulphur dioxide.

Microbial contamination (2.2.9). Total microbial count is not more than 10^3 CFU per g. 1 g is free from *Escherichia coli* and 10 g is free from *Salmonella* and *Shigella*.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined by the following method.

Weigh about 1.0 g in a stainless steel dish weighing about 25 g and with a diameter of 70 mm and a height of 15 mm, fitted with a cover. Add 10 ml of *water* and allow to soak. Heat on a water-bath to form a homogeneous solution and continue heating until most of the water has evaporated. Dry for 2 hours at 105° and for further periods of 30 minutes until two successive weighings do not differ by more than 1 mg (Do not powder sheet gelatin while preparing for this test).

Gelatin intended for use in the preparation of pessaries and suppositories complies with the following additional requirements.

Peroxides. Not more than 10 ppm, determined by using *peroxide test strips*.

Dip a test strip for 1 second into *hydrogen peroxide standard solution (10 ppm H_2O_2)*, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and compare the reaction zone after 15 seconds with the colour scale provided with the test strips used. The colour must match that of the 10 ppm concentration, otherwise the test is invalid.

Weigh 19.9 to 20.1 g of the substance under examination in a beaker and add 79.8 to 80.2 ml of *water*. Stir to moisten all gelatin and allow the sample to stand at room temperature for 1 to 3 hours. Cover the beaker with a watch-glass. Place the beaker for 15 to 25 minutes in a water bath at 63° to 67° to dissolve the sample. Stir the contents of the beaker with a glass rod to achieve a homogeneous solution. Dip a test strip for 1 second into the test solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and compare the reaction zone after 15 seconds with the colour scale provided with the test strips used. Multiply the concentration read from the colour scale by a factor of 5 to calculate the concentration in parts per million of peroxide in the test substance.

Jelly strength (2.4.18). 80.0 per cent to 120.0 per cent of the labeled nominal value.

Storage. Store protected from moisture.

Labelling. The label states the jelly strength.

Hard Gelatin Capsule Shells

Hard Gelatin Capsule Shells are soluble containers for incorporation of drugs, usually in the form of powders, pellets or granules, and are commonly intended for oral

administration. The shells are acted upon by digestive fluids and the filled contents are released. They are composed of gelatin, water and additives such as plasticizers, humectants, surfactants, dispersing agents, flavouring agents, antimicrobial agents, sweetening agents, opacifying agents and one or more colouring agents permitted under the Drugs and Cosmetics Rules, 1945. Ingredients other than colouring agents and opacifying agents comply with the standards of this Pharmacopoeia.

Category. Pharmaceutical aid.

Description. Hard Gelatin Capsule Shells (shells or cases) consist of two cylindrical, telescoping pieces (cap and body), one end of which is rounded and closed and the other, open. Shapes other than cylindrical can also be formed as per requirements. The two pieces are uncoloured or coloured; if coloured, of identical or different colours; transparent or opaque, partially or completely and printed or unprinted or bear other surface markings. The cap overlaps the body and maintains a tight friction closure. The closure may be strengthened by suitable means.

The shells are of various sizes, usually designated by different numbers, 5 being the smallest and 000 the biggest. Shells of sizes 0 to 4 are commonly used. Shells of special shapes, sizes, lengths and designations are also available. The shells are smooth and uniform in size, shape and colour. Guidelines on dimensions with respect to different sizes of commonly used capsules are given in chapter 5.8.

Identification

Boil one capsule shell with 20 ml of *water*, allow to cool and centrifuge. To 5 ml of the supernatant liquid add 1 ml of *picric acid solution* and to another 5 ml add 1 ml of *tannic acid solution*; a precipitate is produced in each case.

Tests

Odour. Keep 100 capsule shells in a well-closed bottle for 24 hours at a temperature between 30° and 40°; the shells do not develop any foreign odour.

NOTE — In order to ensure that the quality of the shells is not affected by temperature and humidity, the capsule shells should be conditioned at a temperature of 25° ± 2° and a relative humidity of 50 ± 5 per cent for not less than 12 hours before conducting the test for Average weight.

Average weight. Weigh 100 capsule shells and determine the average weight of a capsule. The average weight is within ±10 per cent of the target weight shown in Table 1. (As sizes 0 to 4 are commonly used, detailed requirements are included for these sizes only. Requirements for other sizes may be decided upon mutually between the manufacturer of the Hard Gelatin Capsule Shells and the user).

Table 1 - Target weight of capsules

Size	Target weight (mg)
0	96
1	76
2	63
3	50
4	40

Disintegration (2.5.1). 15 minutes, using discs (*NOTE — This test is not applicable to the capsules intended to use for inhalation preparations)*.

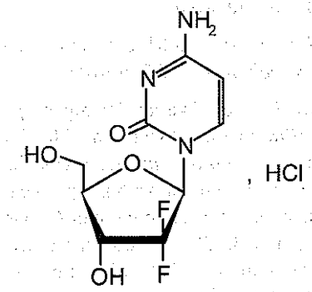
Microbial contamination (2.2.9). Total microbial count is not more than 10^3 CFU per g. 1 g is free from *Escherichia coli* and 10 g is free from *Salmonella* and *Shigella*.

Loss on drying (2.4.19). 12.5 to 16.0 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours or to constant weight.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) the size of the capsule shells; (2) that only permitted colours, if any, have been used; (3) the storage conditions.

Gemcitabine Hydrochloride



$C_9H_{11}F_2N_3O_4,HCl$ Mol Wt. 299.7

Gemcitabine Hydrochloride is Gemcitabine Hydrochloride is 2'-deoxy-2',2'-difluorocytidine hydrochloride.

Gemcitabine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_9H_{11}F_2N_3O_4,HCl$, calculated on as is basis.

CAUTION — Gemcitabine Hydrochloride is cytotoxic; Extra care required to prevent inhaling and exposing the skin to it.

Category. Anticancer.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gemcitabine hydrochloride IPRS* or with the reference spectrum of gemcitabine hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution (Solution A) is clear (2.4.1) and not more intensely colored than reference solution BYS7 (2.4.1).

pH (2.4.24). 2.0 to 3.0, determined in solution A.

Specific optical rotation (2.4.22). +43.0° to +50.0°, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in *water* and dilute to 25.0 ml with *water.*

Test solution (b). Dilute 1.0 ml of test solution (a) to 20.0 ml with *water.*

Reference solution (a). A solution containing 0.02 per cent w/v, each of, *gemcitabine hydrochloride IPRS* and *gemcitabine impurity A IPRS* in *water.* Dilute 1.0 ml of the solution to 100.0 ml with *water.*

Reference solution (b). A 0.01 per cent w/v solution of *gemcitabine hydrochloride IPRS* in *water.*

Reference solution (c). Weigh 10 mg of *gemcitabine hydrochloride IPRS* in a small vial. Add 4.0 ml of a 16.8 per cent w/v solution of *potassium hydroxide* in *methanol*, sonicate for 5 minutes then seal with a cap. The mixture may be cloudy. Heat at 55 ° for a minimum of 6 hour to produce gemcitabine impurity B. Allow to cool, then transfer the entire contents of the vial to a 100-ml volumetric flask by successively washing with a 1.0 per cent v/v solution of *orthophosphoric acid.* Dilute to 100.0 ml with a 1.0 per cent v/v solution of *orthophosphoric acid* and mix.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 13.8 g of *sodium dihydrogen phosphate monohydrate* in 900 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and dilute to 1000 ml with *water*,

B. *methanol*,

- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobil phase B (per cent v/v)
0	97	3
8	97	3
13	50	50
20	50	50
25	97	3

Name	Relative retention time
Gemcitabine impurity A[1]	0.4
Gemcitabine impurity B[2]	0.7
Gemcitabine (Retention time: about 8 minutes)	1.0

[1]cytosine,

[2]gemcitabine α-anomer.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to gemcitabine impurity B and gemcitabine is not less than 8.0.

Inject reference solution (a) and test solution (a). In the chromatogram obtained with test solution (a), the area of any secondary peak is not more than the area of the peak due to gemcitabine in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the peak due to gemcitabine in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the peak due to gemcitabine in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). Dissolve 2.0 g in 20.0 ml of *water.* 12 ml of the solution complies with the limit test for heavy metals, Method D (10 ppm), using 10.0 ml of *lead standard solution (1 ppm).*

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 1.0 g in a platinum crucible.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances with the following modifications.

- mobile phase: a buffer solution prepared by dissolving 13.8 g of *sodium dihydrogen phosphate monohydrate* in 900 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and dilute to 1000 ml with *water*,

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to gemcitabine impurity B and gemcitabine is not less than 8.0.

Inject reference solution (b) and test solution (b).

Calculate the content of $C_9H_{12}ClF_2N_3O_4$.

Gemcitabine Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.05 Endotoxin Unit per mg of gemcitabine.

Storage. Store protected from moisture.

Gemcitabine Injection

Gemcitabine Injection is sterile material consisting of Gemcitabine Hydrochloride with or without auxiliary substances. It is filled in sealed container.

Gemcitabine Hydrochloride Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of gemcitabine, $C_9H_{11}F_2N_3O_4$.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

CAUTION: Gemcitabine Hydrochloride is cytotoxic; Extra care required to prevent inhaling and exposing the skin to it.

Usual strengths. 40 mg per ml.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powder for Injections) and with the following requirements.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution (b).

Tests

pH (2.4.24). 2.7 to 3.3, determined in a 4.0 per cent w/v solution in 0.9 per cent w/v solution of *sodium chloride.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve a quantity of powder to obtain a solution containing 0.2 per cent w/v of gemcitabine in *water.*

Test solution (b). Dilute 1.0 ml of test solution (a) to 20.0 ml with *water.*

Reference solution (a). A solution containing 0.02 per cent w/v, each of, *gemcitabine hydrochloride IPRS* and *gemcitabine impurity A IPRS* in *water.* Dilute 1.0 ml of the solution to 100.0 ml with *water.*

Reference solution (b). A 0.01 per cent w/v solution of *gemcitabine hydrochloride IPRS* in *water.*

Reference solution (c). Weigh 10 mg of the *gemcitabine hydrochloride IPRS* in a small vial. Add 4.0 ml of a 16.8 per cent w/v solution of *potassium hydroxide* in *methanol*, sonicate for 5 minutes then seal with a cap. The mixture may be cloudy. Heat at 55° for a minimum of 6 hour to produce gemcitabine impurity B. Allow to cool, then transfer the entire contents of the vial to a 100.0 ml volumetric flask by successively washing with 1.0 per cent v/v solution of *orthophosphoric acid* and dilute to 100.0 ml with a 1.0 per cent *v/v* solution of *orthophosphoric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 13.8 g of *sodium dihydrogen phosphate monohydrate* in 900 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and dilute to 1000 ml with *water*,

B. *methanol*,

- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobil phase B (per cent v/v)
0	97	3
8	97	3
13	50	50
20	50	50
25	97	3

Name	Relative retention time
Gemcitabine impurity A[1]	0.4
Gemcitabine impurity B[2]	0.7
Gemcitabine (retention time: about 8 minutes)	1.0

[1]cytosine,

[2]gemcitabine α-anomer.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to gemcitabine impurity B and gemcitabine is not less than 8.0.

Inject reference solution (a) and test solution (a). In the chromatogram obtained with test solution (a), the area of any peak corresponding to gemcitabine impurities A and B is not more than the area of the peak due to gemcitabine in the

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 20 capsules. Disperse a quantity of powder containing 0.15 g of Gemfibrozil with 50 ml of *methanol*, shake on a mechanical shaker for 10 minutes, add 20 ml of *water*, 1 ml of *glacial acetic acid* and dilute to 100.0 ml with *methanol*, filter. Dilute 1.0 ml of the filtrate to 5.0 ml with the mobile phase.

Reference solution (a). Dissolve 30 mg of *gemfibrozil IPRS* in 80 ml of *methanol*, add 1 ml of *glacial acetic acid* and dilute to 100.0 ml with *water*.

Referenc solution (b). A 0.01 per cent w/v solution of *gemfibrozil methyl ester IPRS* in a solution prepared by diluting 1 volume of the test solution to 3 volumes with the mobile phase.

Chromatographic system

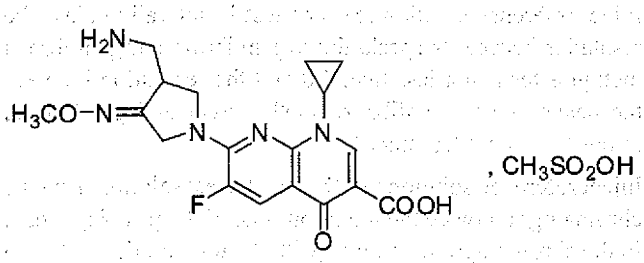
- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 1 volume of *glacial acetic acid*, 19 volumes of *water* and 80 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to gemfibrozil and gemfibrozil methyl ester is not less than 4.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{22}O_3$ in the capsules.

Gemifloxacin Mesylate



$C_{19}H_{24}FN_5SO_7$ Mol. Wt. 485.5

Gemifloxacin Mesylate is 7-[3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulphonate.

Gemifloxacin Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{24}FN_5SO_7$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. An off white to light brown coloured powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gemifloxacin mesylate IPRS* or with the reference spectrum of gemifloxacin mesylate.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *water* and 20 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of solvent mixture.

Reference solution. Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Cosmosil C18),
- column temperature: 45°,
- mobile phase: A. a solution containing 2.0 g of *ammonium acetate* and 3.5 g of *sodium perchlorate* in 650 ml of *water*, adjusted to pH 2.2 with *ortho-phosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	81	19
25	81	19
35	47	53
40	47	53
45	81	19
50	81	19

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution

(1.0 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). 4.0 per cent to 7.0 per cent, determined on 0.4 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *water* and 20 volumes of *acetonitrile.*

Test solution (a). Dissolve 50 mg of the substance under examination in 100.0 ml of the solvent mixture.

Test solution (b). Dilute 5.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *gemifloxacin mesylate IPRS* in the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Cosmosil C18),
- column temperature: 45°,
- mobile phase: a mixture of 70 volumes of buffer solution prepared by dissolving 2.0 g of *ammonium acetate* and 3.5 g of *sodium perchlorate* in 650 ml *water*, adjusted to pH 2.2 with *orthophosphoric acid*, and 30 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and test solution (b).

Calculate the content of $C_{19}H_{24}FN_5SO_7$.

Storage. Store protected from light and moisture.

Gemifloxacin Tablets

Gemifloxacin Mesylate Tablets

Gemifloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of gemifloxacin, $C_{18}H_{20}FN_5O_4$.

Usual strength. 320 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with *water.*

Reference solution. A 0.047 per cent w/v solution of *gemifloxacin mesylate IPRS* in the dissolution medium. Dilute 5 ml of the solution to 50 ml with *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 mm) (Such as Hypersil BDS C18),
- column temperature: 45°,
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 6.0 g of *ammonium acetate* and 10.5 g of *sodium perchlorate monohydrate* in 1950 ml of *water*, adjusted to pH 2.2 with *orthophosphoric acid* and 25 volumes of *acetonitrile,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{20}FN_5O_4$ in the tablet.

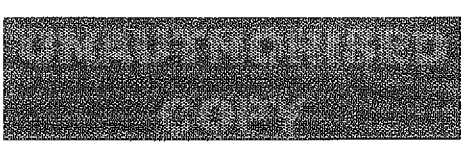
Q. Not less than 70 per cent of the stated amount of $C_{18}H_{20}FN_5O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *water* and 20 volumes of *acetonitrile.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 20 mg of the gemifloxacin with 100.0 ml of the solvent mixture, filter.

Reference solution (a). A 0.025 per cent w/v solution of *gemifloxacin mesylate IPRS* in the solvent mixture.



Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 mm) (Such as phenomenex Luna C18),
- mobile phase: A. buffer solution prepared by dissolving 6.0 g of *ammonium acetate* and 10.5 g of *sodium perchlorate monohydrate* in 1950 ml of *water*, adjusted to pH to 2.2 with *orthophosphoric acid*,

  B. *acetonitrile*,

  C. *tetrahydrofuran*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Mobile phase C (per cent v/v)
0	92	5	3
30	42	55	3
32	92	5	3
40	92	5	3

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and the sum of areas of all the secondary peaks is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 100 mg of gemifloxacin, in *water* and dilute to 100.0 ml with *water* and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *water*.

Reference solution. A 0.01 per cent w/v solution of *gemifloxacin mesylate IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 mm) (Such as Hypersil BDS C18),
- column temperature: 45°,
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 6.0 g of *ammonium acetate* and 10.5 g of *sodium perchlorate monohydrate* in 1950 ml of *water*, adjusted to pH 2.2 with *orthophosphoric acid* and 25 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

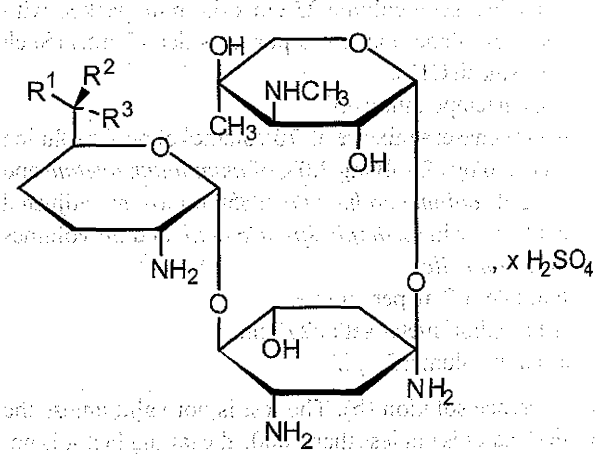
Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{20}FN_5O_4$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of Gemifloxacin.

Gentamicin Sulphate



Gentamycin	R^1	R^2	R^3
C_1	CH_3	$NHCH_3$	H
C_2	CH_3	NH_2	H
C_{1a}	H	NH_2	H
C_{2a}	H	NH_2	CH_3

Gentamicin Sulphate is a mixture of the sulphates of antimicrobial substances produced by *Micromonospora purpurea*.

Gentamicin Sulphate has a potency of not less than 590 µg of gentamicin per mg, calculated on the dried basis.

Category. Antibacterial.

Description. A white or almost white powder; hygroscopic.

Identification

Tests A and B may be omitted if tests C and D are carried out. Test C may be omitted if tests A, B and D are carried out.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. The lower layer obtained by shaking together equal volumes of *strong ammonia solution*, *chloroform* and *methanol* and allowing to separate.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of *water*.

Reference solution. A 0.5 per cent w/v solution of *gentamicin sulphate IPRS* in *water*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *ethanolic ninhydrin solution* and heat at 110° for 5 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. Dissolve 10 mg in 1 ml of *water* and add 5 ml of a 40 per cent w/v solution of *sulphuric acid*. Heat on a water-bath for 10 minutes, cool and dilute to 25 ml with *water*. When examined in the range 240 nm to 330 nm (2.4.7), the resulting solution shows no absorption maximum.

C. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

D. Gives reaction (A) of sulphates (2.3.1).

Tests

Appearance of solution. A 4.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions (2.4.1).

pH (2.4.24). 3.5 to 5.5, determined in solution A.

Specific optical rotation (2.4.22). +107° to +121°, determined in a 10.0 per cent w/v solution.

Composition of gentamicin sulphate. Determine by liquid chromatography (2.4.14).

Test solution. Add 5 ml of *methanol* and 4 ml of *phthalaldehyde reagent* to 10 ml of a 0.1 per cent w/v solution of the substance under examination in *water*, mix, add sufficient *methanol* to produce 25 ml, heat on a water-bath at 60° for 15 minutes and cool. If the solution is not used immediately, cool to 0° and use within 4 hours.

Reference solution. Prepare in the same manner as the test solution but using 10 ml of a 0.1 per cent w/v solution of *gentamicin sulphate IPRS* in place of the solution of the substance under examination.

Chromatographic system

- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution containing 0.55 per cent w/v of *sodium heptanesulphonate monohydrate* in a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of *glacial acetic acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between any two peaks is not less than 1.25, the capacity factor determined from the gentamicin C_1 peak is between 2 and 7, the column efficiency determined from the gentamicin C_2 peak is not less than 1200 theoretical plates.

Inject the reference solution and the test solution. The elution order is gentamicin C_1, gentamicin C_{1a}, gentamicin C_{2a}, and gentamicin C_2. Calculate the content of gentamicin C_1, gentamicin C_{1a}, gentamicin C_{2a}, and gentamicin C_2 in the portion of gentamicin sulphate taken by the formula:

$$\frac{100 \times r_f}{r_s}$$

in which r_f is the peak area response corresponding to the particular gentamicin; and r_s is the sum of the area responses of all four peaks: the content of gentamicin C_1 is between 25 per cent and 50 per cent, the content of gentamicin C_{1a} is between 10 per cent and 35 per cent, and the sum of the contents of gentamicin C_{2a} and gentamicin C_2 is between 25 per cent and 55 per cent.

Sulphate. 32.0 to 35.0 per cent of SO_4, calculated on the dried basis, determined by the following method. Dissolve 0.25 g in 100 ml of *distilled water*, adjusted to pH 11 with *strong ammonia solution* and add 10 ml of *0.1 M barium chloride*. Titrate with *0.1 M disodium edetate* using 0.5 mg of *metalphthalein* as indicator; when the colour of the solution begins to change add 50 ml of *ethanol (95 per cent)* and continue the titration until the violet-blue colour disappears. Perform a blank determination and make any necessary correction.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of sulphate, SO_4.

Methanol. Not more than 1.0 per cent w/w. Determine by gas chromatography (2.4.13).

Test solution (a). A 25 per cent w/v solution of the substance under examination.

Test solution (b). A solution containing 25 per cent w/v of the substance under examination and 0.25 per cent v/v of *1-propanol* (internal standard).

Reference solution. A solution containing 0.25 per cent v/v of *methanol* and 0.25 per cent v/v of the internal standard.

Chromatographic system

- a column 1.5 m x 4 mm, packed with porous polymer beads (80 to 100 mesh)(Such as Porapak Q),
- temperature:
  column. constant at a point between 120° and 140°,
  inlet port and detector. 50° higher than column temperature,
- flow rate: constant at 30 to 40 ml per minute of the carrier gas.

Calculate the percentage w/w of methanol taking 0.792 g as its weight per ml (2.4.29) at 20°.

Sulphated ash (2.3.18). Not more than 1.0 per cent, determined on 0.5 g.

Loss on drying. Not more than 18.0 per cent, determined on 1.0 g by drying in an oven at 110°, at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics (2.2.10), and express the result in µg of gentamicin per mg.

Gentamicin Sulphate intented for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.67 Endotoxin Units per mg of gentamicin.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store protected from moisture. If it is intended for use in the manufacture of parenteral or ophthalmic preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the potency in terms of µg of gentamicin per mg; (2) whether or not the contents are intended for use in the manufacture of parenteral or ophthalmic preparations.

Gentamicin Cream

Gentamicin Sulphate Cream

Gentamicin Cream is a viscous oil-in-water emulsion containing Gentamicin Sulphate dissolved in the aqueous phase.

Gentamicin Cream contains not less than 90.0 per cent and not more than120.0 per cent of the stated amount of gentamicin.

Usual strengths. 0.1 per cent w/w and 0.3 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.6), coating the plate with *silica gel G*.

Mobile phase. The lower layer obtained by shaking equal volumes of *strong ammonia solution*, *chloroform* and *methanol* and allowing to separate.

Test Solution. Disperse a quantity of the cream containing 7.5 mg of Gentamicin with 20 ml of *chloroform*, extract with 10 ml of *water* and use the aqueous layer.

Reference Solution. A 0.075 per cent w/v solution of *gentamicin sulphate IPRS* in *water*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with *ethanolic ninhydin solution* and heat at 105° for 2 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

Tests

Composition of Gentamicin Sulphate. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the cream containing 15 mg of Gentamicin in 10 ml of *chloroform*, add 10 ml of a 0.25 per cent w/v solution of *sodium tetraborate*, shake vigorously, centrifuge and separate the aqueous layer. Repeat the extraction with two quantities, each of 5 ml of *sodium tetraborate solution*, combined the aqueous extract and dilute to 25.0 ml with the same solution and filter. To 10 ml of the resulting solution add 5 ml of *methanol*, swirl and add 4 ml of *phthaladehyde reagent*, mix, add sufficient *methanol* to produce 25.0 ml, heat on a water-bath at 60° for 15 minutes and cool.

Reference solution. Prepare in the same manner as the test solution but using 10 ml of a 0.065 per cent w/v solution of *gentamicin sulphate IPRS* in place of the solution of the preparation under examination. To 10 ml of the resulting solution add 5 ml of *methanol* swirl and add 4 ml of *phthaladehyde reagent*, mix, add sufficient *methanol* to produce 25.0 ml, heat on a water-bath at 60° for 15 minutes and cool.

NOTE — If the solution is not used immediately, cool to 0° and use within 4 hours.

Chromatographic system
- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: *0.025 M sodium heptanesulphonate monohydrate* in a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of *glacial acetic acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- injection volume: 20 µl.

If necessary, adjust the *methanol* content of the mobile phase, so that in the chromatogram obtained with the reference solution, the retention time of the component C_2 is 10 to 20 minutes and the peaks are well separated with relative retention times of about 0.13 (reagent), 0.27 (component C_1), 0.65 (component C_{1a}), 0.85 (component C_{2a}) and 1.00 (component C_2).

Adjust the sensitivity and the volume of reference solution injected so that the height of the peak due to component C_1 is about 75 per cent of the full-scale deflection on the recorder. Plot a horizontal baseline on the chromatogram from the level portion of the curve immediately prior to the reagent peak. Measure the peak height above this baseline for each component. Repeat the procedure with the test solution. The test is not valid unless the resolution between the peaks due to C_{2a} and C_2 is not less than 1.3.

From the peak areas in the chromatogram obtained with the reference solution and the proportions of the components declared for *gentamicin sulphate IPRS*, calculate the response factors for components C_1, C_{1a}, C_{2a} and C_2. From these response factors and peak areas in the chromatogram obtained with the test solution, calculate the proportions of components C_1, C_{1a}, C_{2a} and C_2 in the cream. The proportion are with in the following limits C_1, 25.0 to 50.0 per cent; C_{1a}, 10.0 to 35.0 per cent; C_2+ C_{2a} 25.0 to 55.0 per cent.

Other tests. Complies with the tests stated under Cream.

Assay. Weigh a quantity of the cream containing 3 mg of Gentamicin, dissolve in 20 ml of *chloroform*, shake vigorously with 75 ml of *phosphate buffer pH 8.0* and allow to separate. Dilute 10 ml of the aqueous layer to 50 ml with *phosphate buffer pH 8.0*.

Carry out the microbiological assay of antibiotics (2.2.10).

Calculate the content of gentamicin in the cream, taking each 1000 Units found to be equivalent to 1 mg of gentamicin.

Labelling. The label states the quantity of active ingredient in terms of the equivalent amount of gentamicin.

Gentamicin Eye Drops

Gentamicin Sulphate Eye Drops

Gentamicin Eye Drops are a sterile solution of Gentamicin Sulphate in Purified Water.

Gentamicin Eye Drops contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of gentamicin.

Usual strength. 0.3 per cent w/v of gentamicin.

Identification

A. Determine by thin-layer chromatography (2.4.6), coating the plate with *silica gel G*.

Mobile phase. The lower layer obtained by shaking together equal volumes of *strong ammonia solution*, *chloroform* and *methanol* and allowing to separate.

Test solution. A volume of the eye drops containing 60 µg of gentamicin.

Reference solution. Dissolve 0.1 mg of *gentamicin sulphate IPRS* in a volume of *water* equivalent to the volume of the eye drops used.

Apply to the plate the specified volumes of each solution. After development, dry the plate in air, spray with *ethanolic ninhydrin solution* and heat at 110° for 5 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.5 to 7.5.

Composition of gentamicin sulphate. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the eye drops with *water* to contain 0.045 per cent w/v of gentamicin. To 10 ml of the resulting solution add 5 ml of *methanol*, swirl and add 4 ml of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 ml, heat on a water-bath at 60° for 15 minutes and cool.

Reference solution. Prepare in the same manner as the test solution but using 10 ml of a 0.065 per cent w/v solution of *gentamicin sulphate IPRS* in place of the solution of the preparation under examination.

Chromatographic system
- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: *0.025 M sodium heptanesulphonate monohydrate* in a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of *glacial acetic acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- injection volume: 5 µl.

If necessary, adjust the methanol content of the mobile phase so that in the chromatogram obtained with the reference solution the retention time of component C_2 is 10 to 20 minutes and the peaks are well separated with relative retention times of about 0.13 (reagent), 0.27 (component C_1), 0.65 (component C_{1a}), 0.85 (component C_{2a}) and 1.00 (component C_2).

Adjust the sensitivity and the volume of reference solution injected so that the height of the peak due to component C_1 is about 75 per cent of the full-scale deflection on the recorder. Plot a horizontal baseline on the chromatogram from the level portion of the curve immediately prior to the reagent peak. Measure the peak height above this baseline for each component. Repeat the procedure with the test solution. The test is not valid unless the resolution between the peaks due to components C_{2a} and C_2 is not less than 1.3.

From the peak areas in the chromatogram obtained with the reference solution and the proportions of the components declared for *gentamicin sulphate IPRS*, calculate the response factors for components C_1, C_{1a}, C_{2a} and C_2. From these response factors and peak areas in the chromatogram obtained with the test solution, calculate the proportions of components C_1, C_{1a}, C_{2a} and C_2 in the eye drops. The proportion are within the following limits. C_1, 25.0 to 50.0 per cent; C_{1a}, 10.0 to 35.0 per cent; $C_2 + C_{2a}$, 25.0 to 55.0 per cent.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by the microbiological assay of antibiotics (2.2.10) on a solution prepared in the following manner.

Dilute a volume of the eye drops containing about 15 mg of gentamicin to 50.0 ml with sterile *phosphate buffer pH 8.0* and dilute 10.0 ml of the resulting solution to 50.0 ml with the same solvent.

Calculate the content of gentamicin in the eye drops, taking each 1000 Units found to be equivalent to 1 mg of gentamicin.

Labelling. The label states the quantity of active ingredient in terms of the equivalent amount of gentamicin.

Gentamicin Injection

Gentamicin Sulphate Injection

Gentamicin Injection is a sterile solution of Gentamicin Sulphate in Water for Injection.

Gentamicin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of gentamicin.

Usual strengths. The equivalent of 10 mg per ml and 40 mg per ml of gentamicin.

Description. A clear, colourless to pale-yellow solution.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. The lower layer obtained by shaking together equal volumes of *13.5 M ammonia*, *chloroform* and *methanol* and allowing to separate.

Test solution. Dilute a volume of the injection with sufficient *water* to produce a solution containing 0.5 per cent w/v of gentamicin.

Reference solution. A 0.8 per cent w/v solution of *gentamicin sulphate IPRS* in *water*.

Apply to the plate 6 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air, spray with *ethanolic ninhydrin solution* and heat at 105° for 2 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 5.0.

Composition of gentamicin sulphate. Determine by liquid chromatography (2.4.14).

Test solution. Add 5 ml of *methanol* to 10 ml of a solution prepared by diluting a suitable volume of the injection with *water* to contain the equivalent of 0.045 per cent w/v of gentamicin, swirl and add 4 ml of *phthalaldehyde reagent*, mix, add sufficient *methanol* to produce 25 ml, heat on a water-bath at 60° and cool. If the solution is not used immediately, cool at 0° and use within 4 hours.

Reference solution. Prepare in the same manner as the test solution but using 10 ml of a 0.065 per cent w/v solution of *gentamicin sulphate IPRS* in place of the solution of the injection under examination.

Chromatographic system

- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: *0.025 M sodium heptanesulphonate monohydrate* in a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of *glacial acetic acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- injection volume: 5 µl.

If necessary, adjust the methanol content of the mobile phase so that in the chromatogram obtained with the reference solution the retention time of component C_2 is 10 to 20 minutes and the peaks are well separated with relative retention times of about 0.13 (reagent), 0.27 (component C_1), 0.65 (component C_{1a}), 0.85 (component C_{2a}) and 1.00 (component C_2).

Adjust the sensitivity and the volume of reference solution injected so that the height of the peak due to component C_1 is about 75 per cent of full-scale deflection on the recorder. Plot a horizontal baseline on the chromatogram from the level portion of the curve immediately prior to the reagent peak. Measure the peak height above this baseline for each component. Repeat the procedure with the test solution. The test is not valid unless the resolution factor between the peaks due to components C_{2a} and C_2 is not less than 1.3.

From the peak areas in the chromatogram obtained with the reference solution and the proportions of the components declared for gentamicin sulphate RS, calculate the response factors for components C_1, C_{1a}, C_{2a} and C_2. From these response factors and peak areas in the chromatogram obtained with the test solution, calculate the proportions of components C_1, C_{1a}, C_{2a} and C_2 in the injection. The proportions are within the following limits. C_1, 25.0 to 50.0 per cent; C_{1a}, 10.0 to 35.0 per cent; $C_2 + C_{2a}$, 25.0 to 55.0 per cent.

Bacterial endotoxins (2.2.3). Not more than 1.67 Endotoxin Units per mg of gentamicin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by the microbiological assay of antibiotics (2.2.10), and express the result in mg of gentamicin per ml.

Calculate the content of gentamicin in the injection, taking each 1000 Units found to be equivalent to 1 mg of gentamicin.

Labelling. The label states the strength in terms of the equivalent amount of gentamicin in a suitable dose-volume.

Gentamicin Ointment

Gentamicin Sulphate Ointment

Gentamicin Ointment is a dispersion of Gentamicin Sulphate in microfine powder in white soft paraffin or other suitable anhydrous greasy basis.

Gentamicin Ointment contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of gentamicin.

Usual strengths. 0.1 per cent w/w and 0.3 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.6), coating the plate with *silica gel G*.

Mobile phase. The lower layer obtained by shaking equal volumes of *strong ammonia solution*, *chloroform* and *methanol* and allowing to separate.

Test solution. Mix a quantity of the ointment containing 7.5 mg of Gentamicin with 20 ml of *chloroform*, extract with 10 ml of *water* and use the aqueous layer.

Reference solution. A 0.075 per cent w/v solution of *gentamicin sulphate IPRS* in *water*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, spray with *ethanolic ninhydin solution* and heat at 105° for 2 minutes. The three principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

B. In the test for Composition of gentamicin sulphate, the four principal peaks in the chromatogram obtained with the test solution correspond to the four peaks in the chromatogram obtained with the reference solution.

Tests

Composition of Gentamicin Sulphate. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the ointment containing 20 mg of Gentamicin in 10 ml of *chloroform*, add 20 ml of a 0.25 per cent w/v solution of *sodium tetraborate*, shake vigorously, centrifuge and separate the aqueous layer. Filter and dilute to 50.0 ml with *water*. To 10 ml of the resulting solution add 5 ml of *methanol*, swirl and add 4 ml of *phthaladehyde reagent*, mix, add sufficient *methanol* to produce 25.0 ml, heat on a water-bath at 60° for 15 minutes and cool.

Reference solution. Prepare in the same manner as the test solution but using 10 ml of a 0.065 per cent w/v solution of *gentamicin sulphate IPRS* in place of the solution of the preparation under examination. To 10 ml of the resulting solution add 5 ml of *methanol*, swirl and add 4 ml of *phthaladehyde reagent*, mix, add sufficient *methanol* to produce 25.0 ml, heat on a water-bath at 60° for 15 minutes and cool.

NOTE — If the solution is not used immediately, cool to 0° and use within 4 hours.

Chromatographic system

- a stainless steel column 10 to 12.5 cm x 4.6 to 5 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: *0.025 M sodium heptanesulphonate monohydrate* in a mixture of 70 volumes of *methanol*, 25 volumes of *water* and 5 volumes of *glacial acetic acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 330 nm,
- injection volume: 20 µl.

If necessary, adjust the *methanol* content of the mobile phase, so that in the chromatogram obtained with the reference solution, the retention time of the component C_2 is 10 to 20 minutes and the peaks are well separated with relative retention times of about 0.13 (reagent), 0.27 (component C_1), 0.65 (component C_{1a}), 0.85 (component C_{2a}) and 1.00 (component C_2).

Adjust the sensitivity and the volume of reference solution injected so that the height of the peak due to component C_1 is about 75 per cent of the full-scale deflection on the recorder. Plot a horizontal baseline on the chromatogram from the level portion of the curve immediately prior to the reagent peak. Measure the peak height above this baseline for each component. Repeat the procedure with the test solution. The test is not valid unless the resolution between the peaks due to C_{2a} and C_2 is not less than 1.3.

From the peak areas in the chromatogram obtained with the reference solution and the proportions of the components declared for *gentamicin sulphate IPRS*, calculate the response factors for components C_1, C_{1a}, C_{2a} and C_2. From these response factors and peak areas in the chromatogram obtained with the test solution, calculate the proportions of components C_1, C_{1a}, C_{2a} and C_2 in the cream. The proportion are with in the following limits. C_1, 25.0 to 50.0 per cent; C_{1a}, 10.0 to 35.0 per cent; C_2+ C_{2a} 25.0 to 55.0 per cent.

Other tests. Complies with the tests stated under Ointments.

Assay. Weigh a quantity of the ointment containing 4 mg of Gentamicin, dissolve in 50 ml of *chloroform*, extract with three quantities, each of 20 ml, of *sterile phosphate buffer pH 8.0*, combine the extracts and dilute to 100.0 ml with *phosphate buffer pH 8.0*. Dilute 10.0 ml of the resulting solution to 50.0 ml with *phosphate buffer pH 8.0*.

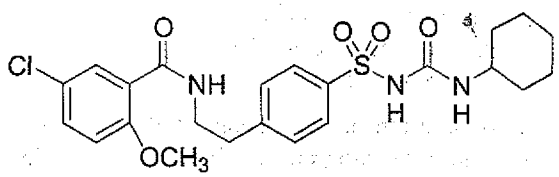
Carry out the microbiological assay of antibiotics (2.2.10).

Calculate the content of gentamicin in the ointment, taking each 1000 Units found to be equivalent to 1 mg of gentamicin.

Labelling. The label states the quantity of active ingredient in terms of the equivalent amount of gentamicin.

Glibenclamide

Glyburide



$C_{23}H_{28}ClN_3O_5S$ Mol. Wt. 494.0

Glibenclamide is 1-{4-[2-(5-chloro-2-methoxybenzamido) ethyl}benzenesulphonyl}-3-cyclohexylurea.

Glibenclamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{23}H_{28}ClN_3O_5S$, calculated on the dried basis.

Category. Hypoglycaemic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glibenclamide IPRS* or with the reference spectrum of glibenclamide

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.01 M methanolic hydrochloric acid* shows an absorption maximum at about 300 nm and a less intense maximum at about 275 nm; absorbance at about 300 nm, about 0.63 and at about 275 nm, about 0.29.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

D. Dissolve 20 mg in 2 ml of *sulphuric acid (96 per cent w/w)*; the solution is colourless and exhibits a blue fluorescence under ultraviolet light at 365 nm. Dissolve about 0.1 g of *chloral hydrate* in the solution; within 5 minutes the colour changes to deep yellow and after about 20 minutes a brownish tinge is produced.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *ethanol (95 per cent)*, prepared with the aid of heat, is clear (2.4.1), and colourless (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of *methanol.*

Reference solution. A 0.00125 per cent w/v solution of *glibenclamide IPRS* in *methanol.*

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3µm),
- column temperature: 35°,
- mobile phase: A. a mixture of a 20 volumes of 10.2 per cent v/v solution of freshly distilled *triethylamine* adjusted to pH 3.0 with *orthophosphoric acid* and 50 volumes of *acetonitrile*, dilute to 1000 ml with *water*,
  B. a mixture of 20 volumes of mobile phase A, 65 volumes of *water* and 915 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	45	55
15	45	55
30	5	95
40	5	95
41	45	55
55	45	55

The relative retention time with reference to glibenclamide for glibenclamide impurity A is about 0.5, for glibenclamide impurity B is about 0.6.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained in the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.4 g, dissolve in 100 ml of *ethanol (95 per cent)* with the aid of heat; titrate with *0.1 M sodium hydroxide* using 1 ml of *dilute phenolphthalein solution* as indicator until a pink colour is obtained. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0494 g of $C_{23}H_{28}ClN_3O_5S$.

Glibenclamide Tablets

Glibenclamide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of glibenclamide, $C_{23}H_{28}ClN_3O_5S$.

Usual strengths. 2.5 mg; 5 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 45 volumes of *chloroform*, 45 volumes of *cyclohexane*, 5 volumes of *glacial acetic acid* and 5 volumes of *ethanol (95 per cent).*

Test solution. Extract a quantity of the powdered tablets containing 20 mg of Glibenclamide with four quantities, each of 5 ml, of a mixture of 20 volumes of *dichloromethane* and 10 volumes of *acetone*, evaporate the combined extracts to dryness at a pressure of 2 kPa and at a temperature not exceeding 40° and dissolve the residue in 4 ml of a mixture of equal volumes of *chloroform* and *methanol.*

Reference solution (a). A 0.5 per cent w/v solution of *glibenclamide IPRS* in the same solvent mixture.

Reference solution (b). Dilute 2 ml of reference solution (a) to 100 ml with the same solvent mixture.

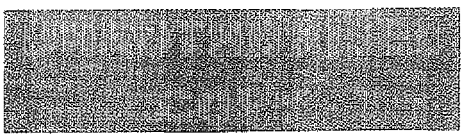
Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following test solution.

Test solution. Disperse one tablet in a mixture of 2 ml of *water* and 20 ml of *methanol,* mix with the aid of ultrasound until fully dispersed and filter.

Other tests. Comply with the tests stated under Tablets.



Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 5 mg of Glibenclamide and disperse with the aid of ultrasound, with a mixture of 2.0 ml of *water* and 20.0 ml of *methanol*. Shake for further 10 minutes, filter, rejecting the first few ml of filterate.

Reference solution. Dissolve 50 mg of *glibenclamide IPRS* in 50 ml of *methanol*, sonicate for 20 minutes. Dilute 1.0 ml of the solution to 4.0 ml with *methanol*. To 20 ml of the solution add 2 ml *water* and mix.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Spherisorb ODS),
- mobile phase: a mixture of 47 volumes of *acetonitrile* and 53 volumes of a 1.36 per cent w/v solution of *potassium dihydrogen orthophosphate*, previously adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{28}ClN_3O_5S$ in the tablets.

Glibenclamide and Metformin Tablets

Glibenclamide and Metformin Hydrochloride Tablets; Glyburide and Metformin Hydrochloride Tablets

Glibenclamide and Metformin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of glibenclamide, $C_{23}H_{28}ClN_3O_5S$ and metformin hydrochloride, $C_4H_{11}N_5,HCl$.

Usual strengths. Glibenclamide, 1.25 mg and Metformin hydrochloride, 250 mg; Glibenclamide, 2.5 mg and Metformin hydrochloride, 250, 400 mg; Glibenclamide, 5 mg and Metformin hydrochloride, 500 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with test solution correspond to the principal peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

For Glibenclamide—

Apparatus No. 2 (Paddle),

Medium. 500 ml of a buffer prepared by dissolving 3.09 g of *boric acid* and 3.73 g of *potassium chloride* in 250 ml of *water*, adjusted to pH 9.5 with *1M sodium hydroxide* and dilute to 1000 ml with *water*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. The filtrate obtained as above. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Weigh and transfer about 12.5 mg of *glibenclamide IPRS* to a 100.0 ml volumetric flask, add 20.0 ml of *acetonitrile*, shake to dissolve and dilute to volume with medium. Dilute 2.0 ml of the solution to 100.0 ml with medium.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50.0 volumes of a buffer solution prepared by dissolving 28.7 g of *monobasic ammonium phosphate* in 1000 ml of *water*, adjusted to pH 5.3 with *1M sodium hydroxide* and 50.0 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 200 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is between 0.8 to 2.0 and relative standard deviation is not more than 2 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{28}ClN_3O_5S$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{23}H_{28}ClN_3O_5S$.

For Metformin Hydrochloride —

Apparatus No. 2 (Paddle),

Medium. 1000 ml, prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjusted to pH 6.8 with *0.2 M sodium hydroxide*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with dissolution medium if necessary, at the maximum at about 232 nm (2.4.7). Calculate the content of $C_4H_{11}N_5,HCl$, in the medium from the absorbance obtained from a solution of known concentration of *metformin hydrochloride IPRS* prepared by dissolving in the medium.

Calculate the content of $C_4H_{11}N_5,HCl$ in the medium.

Q: Not less than 75 per cent of the stated amount of $C_4H_{11}N_5HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

For Glibenclamide —

Solvent mixture. A mixture of equal volumes of *acetonitrile* and *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 2.5 mg of Glibenclamide, disperse in the solvent mixture, dilute to 100.0 ml with the same solvent and filter.

Reference solution (a). Dissolve 12.5 mg of *glibenclamide IPRS* in 25.0 ml of *acetonitrile* and dilute to 50.0 ml with *water.* Dilute 10.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml from reference solution (a) to 100.0 ml with the solvent mixture.

Reference silution(c). A solution containing 0.0025per cent w/v of *glibenclamide related compound A IPRS* in the solvent mixture. Dilute 50 µl in 50 ml with reference solution (a).

Reference solution(d). A solution containing 50 mg of *metformin hydrochloride IPRS* dilute with 10 ml of reference solution (c).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 28.8 g of *monobasic ammonium phosphate* in 800 ml of *water* adjusted to pH 5.3 with *1M sodium hydroxide* and dilute to 1000 ml with *water* and 40 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 100 µl.

Name	Relative retention time	Correction factor
Glibenclamide related compound A[1]	0.30	0.83
Glibenclamide	1	---
Unknown	1	---

[1](4-[2-(5-Chloro-2-methoxybenzamido)ethyl]benzenesulfonamide.

Inject reference solution (d). The relative retation time with reference to glibenclamide for glibenclamide related compound A is 0.3. The test is not valid unless the capacity factor is not less than 7.0, column efficiency is not less than 3000 theoretical plates.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of peak due to glibenclamide related compound A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The area of any secondary peak is not more than 0.2 times of the area of principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The sum of the areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) excluding peak due to glibenclamide related compound A. Ignore any peak with an area 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

For Metformin Hydrochloride —

Solvent mixture. A mixture of 1 volume of *acetonitrile* and 40 volumes of *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of Metformin Hydrochloride, disperse in the solvent mixture, dilute to 100.0 ml with the same solvent and filter.

Reference solution (a). A solution containing 0.025 per cent w/v solution of *metformin hydrochloride IPRS* in solvent mixture.

Reference solution (b). A solution containing 0.0025 per cent w/v solution each of *metformin hydrochloride related compound B IPRS* and *metformin hydrochloride related compound C IPRS* in the solvent mixture.

Reference solution(c). Dilute 0.5 ml from reference solution (b) to 50.0 ml with the reference solution (a).

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 90 volumes of a buffer solution prepared by dissolving 1.0 g each of *sodium heptanesulphonate* and *sodium chloride* in 1800 ml of *water,* adjusted to pH 3.8 with *0.06 M orthophosphoric acid* and dilute to 2000 ml with *water* and 10 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume: 5 µl.

The relative retention time for metformin related compound B, metformin and metformin related compound C is about 0.86, 1.0 and 2.1 to 2.3 respectively.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to metformin related compound B and metformin is not less than 1.5.

Inject the test solution. The area of any secondary peak is not more than 0.1 per cent and the sum of areas of all the secondary

peaks is not more than 0.5 per cent, calculated by area normalization and ignore any peak with an area less than 0.05 per cent of the principal peak (0.05 per cent).

Uniformity of Content (2.5.4). Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of equal volumes of *acetonitrile* and *water.*

Test solution. Disperse one tablet with 20 ml of the solvent mixture shake for 30 minutes and dilute suitably to obtain a solution containing 0.0025 per cent w/v of glibenclamide.

Reference solution. A 0.0025 per cent w/v solution of *glibenclamide IPRS* in the solvent mixture.

Carry out the chromatographic procedure described under Related substances.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{28}ClN_3O_5S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

For Glibenclamide —

Solvent mixture. A mixture of equal volumes of *acetonitrile* and *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 2.5 mg of Glibenclamide, disperse in the solvent mixture, dilute to 100.0 ml with the same solvent and filter.

Reference solution. Weigh and dissolve 12.5 mg of *glibenclamide IPRS* in 25.0 ml of *acetonitrile* and dilute to 50.0 ml with *water.* Dilute 2.0 ml of the solution to 20.0 ml with the solvent mixture.

Use the chromatographic system as described under Related substances of glyburide.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{28}ClN_3O_5S$ in the tablets.

For Metformin Hydrochloride —

Solvent mixture. A mixture of 1 volume of *acetonitrile* and 40 volumes of *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of Metformin Hydrochloride, disperse in the solvent mixture , dilute to 100.0 ml with the same solvent and filter.

Reference solution. A 0.025 per cent w/v solution of *metformin hydrochloride IPRS* in the solvent mixture.

Use the chromatographic system as described under Related substances of metformin .

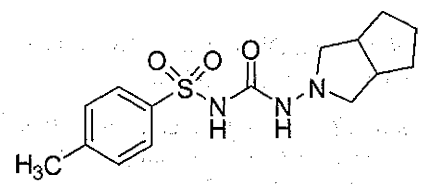
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent for metformin peak.

Inject the reference solution and the test solution.

Calculate the content of $C_4H_{11}N_5HCl$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Gliclazide



$C_{15}H_{21}N_3O_3S$ Mol. Wt. 323.4

Gliclazide is 1-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(4-methylphenyl)sulphonyl]urea.

Gliclazide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{15}H_{21}N_3O_3S$, calculated on the dried basis.

Category. Hypoglycaemic.

Description. A white or almost white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gliclazide IPRS* or with the reference spectrum of gliclazide.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. 45 volumes of *acetonitrile* and 55 volumes of *water.*

Test solution. Dissolve 50 mg of the substance under examination in 23 ml of *acetonitrile* and dilute to 50.0 ml with *water.*

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (b). Dissolve 5 mg of the substance under examination and 15 mg of *1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(2-methylphenyl)sulphonyl]urea IPRS (gliclazide impurity F IPRS)* in 23 ml of *acetonitrile* and dilute to 50 ml with *water*. Dilute 1 ml of the solution to 20 ml with the solvent mixture.

Reference solution (c). Dissolve 1.0 mg of *gliclazide impurity F IPRS* in 5 ml of *acetonitrile* and dilute to 10.0 ml with *water*. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.1 volume of *triethylamine*, 0.1 volume of *trifluoroacetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Inject reference solution (b). The relative retention time with reference to gliclazide for gliclazide impurity F is about 0.9. The resolution between the peaks due to gliclazide impurity F and gliclazide is not less than 1.8.

Inject reference solution (a), (c) and the test solution. Run the chromatogram twice the retention time of the principal peak. The area of peak corresponding to gliclazide impurity F is not more than the area of peak obtained in the chromatogram with reference solution (c) (0.1 per cent), the area of any other secondary peak is not more than the principle peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all other secondary peaks is not more than twice the area of principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Gliclazide impurity B. Determine by liquid chromatography (2.4.14), as described under Related substances.

Test solution. Dissolve 0.4 g of the substance under examination in 2.5 ml of *dimethyl sulphoxide* and dilute to 10.0 ml with *water*. Stir for 10 minutes, store at 4° for 30 minutes and filter.

Reference solution. Dissolve 20 mg of *2-nitroso-octahydrocyclopenta[c]pyrrole IPRS (gliclazide impurity B IPRS)* in 100.0 ml of *dimethyl sulphoxide*. To 1.0 ml of the solution, add 12 ml of *dimethyl sulphoxide* and dilute to 50.0 ml with *water*. To 1.0 ml of the solution, add 12 ml of *dimethyl sulphoxide* and dilute to 50.0 ml with *water*.

Inject 50 µl of the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to impurity B is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (2 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.25 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh 0.25 g, dissolve in 50 ml of *anhydrous acetic acid* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03234 g of $C_{15}H_{21}N_3O_3S$.

Gliclazide Tablets

Gliclazide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of gliclazide, $C_{15}H_{21}N_3O_3S$.

Usual strength. 80 mg.

Identification

Shake a quantity of the powdered tablets containing 0.16 g of Gliclazide with 20 ml of *dichloromethane*, centrifuge and evaporate the supernatant liquid to dryness. The residue complies with the following test. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *gliclazide IPRS* or with the reference spectrum of gliclazide.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 7.4*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary with dissolution medium to obtain a solution containing 12.5 µg per ml of Gliclazide at 226 nm and 290 nm. Correct the absorbance obtained at 226 nm by subtracting the absorbance obtained at 290 nm. Calculate the content of gliclazide, $C_{15}H_{21}N_3O_3S$ in the medium from the absorbances obtained from a solution prepared by dissolving 62 mg of *gliclazide IPRS* in 20 ml of *methanol*, adding sufficient dissolution medium to produce 1000 ml, dilute 1 ml of the solution to 5 ml with the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{15}H_{21}N_3O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. 45 volumes of *acetonitrile* and 55 volumes of *water*.

Test solution. Shake a quantity of the powdered tablets containing about 0.8 g of Gliclazide for 1 hour with 200 ml of *acetonitrile*, filter. Dilute 10.0 ml of the filtrate to 50.0 ml with a mixture of 1 volume of *acetonitrile* and 2 volumes of *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 500.0 ml with the solvent mixture.

Reference solution (b). Dissolve 5.0 mg of *gliclazide IPRS* and 15 mg of *1-(3-azabicyclo[3.3.0]oct-3-yl)-3-o-tolylsulpho-nylurea IPRS* in 25 ml of *acetonitrile*, dilute to 50.0 ml with *water*. Dilute 1.0 ml of the solution to 20.0 ml with the solvent mixture.

Reference solution (c). Dissolve 8.0 mg of *1-(3-azabicyclo[3.3.0]oct-3-yl)-3-o- tolylsulphonylurea IPRS* in 25 ml of *acetonitrile*, dilute to 50 ml with *water*. Dilute 1 ml of the solution to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with endcapped octylsilane bonded to porous silica (4 µm) (Such as Superspher 60 RP 8),
- mobile phase: a mixture of 0.1 volume of *triethylamine*, 0.1 volume of *trifluoroacetic acid*, 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between gliclazide and 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-*o*-tolylsulphonylurea is not less than 1.8.

Inject reference solution (a), (c) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with test solution, the area of peak corresponding to 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-*o*-tolylsulphonylurea is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent); the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of other secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*.

Test solution. Shake a quantity of the powdered tablets containing about 0.8 g of Gliclazide for 1 hour with 200 ml of *acetonitrile*, filter. Dilute 10.0 ml of the filtrate to 200.0 ml with the solvent mixture.

Reference solution (a). Dissolve 40 mg of *gliclazide IPRS* in 10 ml of *acetonitrile* and dilute to 200.0 ml with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *gliclazide IPRS* and 15 mg of *1-(3-azabicyclo[3.3.0]oct-3-yl)-3-o-tolylsulphonyl-urea IPRS* in 25 ml of *acetonitrile*, dilute to 50 ml with *water*. Dilute 1.0 ml of the solution to 20.0 ml with the solvent mixture.

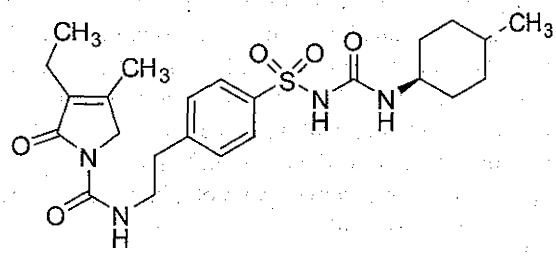
Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-o-tolylsulphonylurea and gliclazide is not less than 1.8.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{21}N_3O_3S$ in the tablets.

Glimepiride



$C_{24}H_{34}N_4O_5S$ Mol. Wt. 490.6

Glimepiride is 1-[[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulphonyl]-3-*trans*-(4-methylcyclohexyl)urea.

Glimepiride contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{24}H_{34}N_4O_5S$, calculated on the anhydrous basis.

Category. Hypoglycaemic.

Descripiton. A white or almost white powder.

Identificaition

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with that *glimepiride IPRS* or with the reference spectrum of glimepiride.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Store the solutions at a temperature not exceeding 12° and for not more than 15 hours.

Solvent mixture. 20 volumes of *water* and 80 volumes of *acetonitrile.*

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). Dissolve the contents of a vial of *glimepiride for system suitability IPRS* (containing Glimepiride impurity B, C and D) in 2.0 ml of the test solution.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (c). A 0.02 per cent w/v solution of *glimepiride IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with endcapped octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 50 volumes of a solution prepared by dissolving 0.5 g of *sodium dihydrogen orthophosphate* in 500 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to glimepiride impurity B and glimepiride impurity C is not less than 4.0. The relative retention time with reference to glimepiride for 3-ethyl-4-methyl-2-oxo-*N*-[2-(4- sulphamoylphenyl)ethyl]-2,3-dihydro-1*H*-pyrrole-1-carboxamide (glimepiride sulphonamide) (glimepiride impurity B) is about 0.2, for methyl [[4-[2-[[(3-ethyl-4-methyl-2-oxo-2, 3-dihydro-1*H*-pyrrol-1-yl)carbonyl] amino] ethyl]phenyl] sulphonyl]carbamate (glimepiride urethane) (glimepiride impurity C) is about 0.3 and for 1-[[3-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-l) carbonyl]amino]ethyl]n phenyl] sulphonyl]-3-(*trans*-4-methylcyclohexyl)urea (glimepiride impurity D) is about 1.1.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to glimepiride impurity B is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent), the area of the peak due to glimepiride impurity D is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of all the secondary peaks other than glimepiride impurity B is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity A. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 10 mg of the substance under examination in 5 ml of *dichloromethane* and dilute to 20.0 ml with the mobile phase.

Reference solution (a). Dilute 0.8 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 2 mg of *glimepiride IPRS* (containing glimepiride impurity A) in 1.0 ml of *dichloromethane* and dilute to 4.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.0 mm, packed with diol silica gel (5 µm),
- mobile phase: a mixture of 1 volume of *anhydrous acetic acid,* 100 volumes of *2-propanol* and 899 volumes of *heptane*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 2.0, where H_p is the height above the baseline of the peak due to impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to glimepiride. The relative retention time with reference to glimepiride for glimepiride impurity A is about 0.9.

Inject reference solution (a) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to 1-[[4-[2-[[(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl]amino]ethyl]phenyl] sulphonyl]-3-(*cis*-4-methylcyclohexyl)urea (glimepiride impurity A) is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent, Method 3, determined by dissolving 0.25 g in 5.0 ml of *dimethylformamide.* Carry out the test on 1.0 ml of the solution. Carry out a blank test.

Assay. Determine by liquid chromatography (2.4.14) as described under test for Related substances with the following modification.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{24}H_{34}N_4O_5S$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Glimepiride Tablets

Glimepiride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of glimepiride, $C_{24}H_{34}N_4O_5S$.

Usual strengths. 1 mg; 2 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of phosphate buffer pH 7.8 prepared by dissolving 0.58 g of *monobasic potassium phosphate* and 8.86 g of *anhydrous dibasic sodium phosphate* in 1000 ml of *water* and adjusted to pH 7.8 with dilute *orthophosphoric acid* or *1 M sodium hydroxide*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay, using injection volume: 50 µl.

Solvent mixture. 50 volumes of *methanol* and 50 volumes of *water*.

Test solution. Use the filtrate, dilute if necessary, with the solvent mixture.

Reference solution. Dissolve a quantity of *glimepiride IPRS* in a mixture of 90 volumes of *acetonitrile* and 10 volumes of *water* to obtain a solution having a concentration of 0.125 mg of glimepiride per ml. Dilute 4.0 ml of the solution to 200.0 ml with the dissolution medium. Dilute 15.0 ml of the solution to 50 ml with the solvent mixture to obtain a final concentration of 0.00075 mg per ml of glimepiride.

Calculate the content of $C_{24}H_{34}N_4O_5S$ in the tablet.

Q. Not less than 75.0 per cent of the stated amount of $C_{24}H_{34}N_4O_5S$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solution.

Solvent mixture. 90 volumes of *acetonitrile* and 10 volumes of *water*.

Test solution. Disperse a quantity of powdered tablets containing about 5 mg of glimepiride in 50.0 ml of the solvent mixture. Centrifuge and use the clear supernatant.

Reference solution (a). A solution containing 0.004 per cent w/v of *glimepiride IPRS* and 0.002 per cent w/v each of *glimepiride sulphonamide (glimepiride impurity B IPRS)* and *glimepiride urethane (glimepiride impurity C IPRS)* in the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 20.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 50 volumes of a solution containing 0.5 g of *monobasic sodium phosphate* in 500 ml of *water*, adjusted to pH 2.1 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to glimepiride impurity B and glimepiride impurity C is not less than 4.0 and the relative standard deviation for replicate injections of glimepiride is not more than 2.0 per cent. The relative retention time with reference to glimepiride for glimepiride impurity B is about 0.2 and for glimepiride impurity C is about 0.3.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak corresponding to glimepiride imputiy B multiplied with correction factor of 0.77 is not more than 2.5 times the area of principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent), the area of any other secondary peak is not more than 0.5 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks other than glimepiride impurity B is not more than the area of principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the sum of areas of all the secondary peaks is not more than 3.5 times the area of principal peak in the chromatogram obtained with reference solution

(b) (3.5 per cent). Ignore any peak with an area less than 0.1 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

Test solution. Disperse one tablet in the solvent mixture and dilute with the solvent mixture to obtain a solution containing 0.01 per cent w/v of glimepiride.

Calculate the content of $C_{24}H_{34}N_4O_5S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solution.

Solvent mixture. 90 volumes of *acetonitrile* and 10 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 10 mg glimepiride in the solvent mixture and dilute with the solvent mixture to obtain a solution containing 0.01 per cent w/v of glimepiride.

Reference solution (a). A solution containing 0.01 per cent w/v of *glimepiride IPRS* and 0.002 per cent w/v each of *glimepiride impurity B IPRS* and *glimepiride impurity C IPRS* in the solvent mixture.

Reference solution (b). A 0.01 per cent w/v solution of *glimepiride IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 50 volumes of a solution containing 0.5 g of *monobasic sodium phosphate* in 500 ml of *water*, adjusted to pH 2.1 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume: 10 µl.

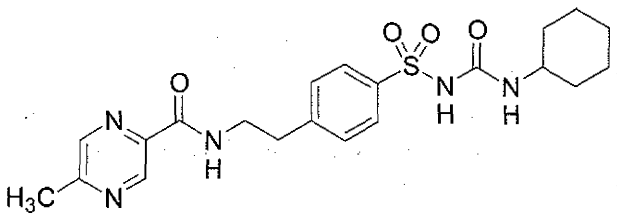
Inject reference solution (a). The test is not valid unless the resolution between the peaks due to glimepiride impurity B and glimepiride impurity C is not less than 1.5 and the relative standard deviation for replicate injections of glimepiride is not more than 2.0 per cent. The relative retention time with reference to glimepiride for glimepiride impurity B is about 0.25 and for glimepiride impurity C is about 0.35.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{24}H_{34}N_4O_5S$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Glipizide



$C_{21}H_{27}N_5O_4S$ Mol. Wt. 445.5

Glipizide is 1-cyclohexyl-3-[[4-[2-[[(5-methylpyrazine-2-yl)carbonyl]amino]ethyl]phenyl]sulphonyl]urea

Glipizide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{27}N_5O_4S$, calculated on the dried basis.

Category. Hypoglycaemic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glipizide IPRS* or with the reference spectrum of glipizide.

B. When examined in the range 220 to 350 nm (2.4.7), a 0.002 per cent solution in *methanol*, shows two maxima, at about 226 nm and 274 nm. The ratio of the absorbance at 226 nm to that at about 274 nm, 2.0 to 2.4 .

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. Equal volumes of *methanol* and *methylene chloride*.

Mobile phase. A mixture of 25 volumes of *anhydrous formic acid*, 25 volumes of *ethyl acetate* and 50 volumes of *methylene chloride*.

Test solution. Dissolve 0.10 g of the substance under examination in 100 ml of solvent mixture.

Reference solution. A 0.10 per cent w/v solution of *glipizide IPRS* in solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v each of *5-methyl-N-[2-(4-sulphamoylphenyl)ethyl] pyrazine-2- carboxamide IPRS (glipizide impurity A IPRS)* and *6-methyl-N-[2-(4-sulphamoylphenyl)ethyl]pyrazine-2- carboxamide IPRS (glipizide impurity D IPRS)* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 17 volumes of *acetonitrile* and 83 volumes of 0.35 per cent w/v solution of *dipotassium hydrogen phosphate*, adjusted to pH 8.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume: 50 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to glipizide impurity A and glipizide impurity D is not less than 2.0. The relative retention time with reference to glipizide for glipizide impurity A is about 0.3, for glipizide impurity D is about 0.4 and for 1-cyclohexyl-3-[[4-[2-[[(6-methylpyrazin-2- yl)carbonyl]amino]ethyl] phenyl] sulphonyl]urea (glipizide impurity E) is about 1.1.

Inject reference solution (a) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to glipizide impurity A is not more 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of the peak due to glipizide impurity D and E is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

Assay. Weigh 0.4 g, dissolve in 50 ml of *dimethylformamide*, add 0.2 ml of *quinaldine red solution*. Titrate with *0.1 M lithium methoxide* until the colour changes from red to colourless. Carry out a blank titration.

1 ml of *0.1 M lithium methoxide* is equivalent to 0.04455 g of $C_{21}H_{27}N_5O_4S$.

Storage. Store protected from moisture.

Glipizide Tablets

Glipizide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of glipizide, $C_{21}H_{27}N_5O_4S$.

Usual strength. 5 mg.

Identification

A. Shake a quantity of the powdered tablets containing 25 mg of Glipizide with 10 ml of *dichloromethane* for 5 minutes, dry over *anhydrous sodium sulphate* and filter. Evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glipizide IPRS* or with the reference spectrum of glipizide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of simulated intestinal fluid (without pancreatin) prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate in* 250 ml *water*, mix and add 77 ml of *0.2 M sodium hydroxide*, add 500 ml of *water*, adjusted to pH 6.8 with *0.2 M sodium hydroxide* or *0.2 M hydrochloric acid* and dilute to 1000 ml with *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, dilute suitably if necessary with the medium, at the maximum at about 276 nm (2.4.7). Calculate the content of $C_{21}H_{27}N_5O_4S$ in the medium from the absorbance obtained from a solution of known concentration of *glipizide IPRS* in the dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{21}H_{27}N_5O_4S$.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 13.8 g of *monobasic sodium phosphate* in *water* and dilute to 1000 ml with *water*, adjusted to pH 6.0 with *2 M sodium hydroxide*.

Test solution. Disperse a quantity of the powdered tablets containing 5 mg of Glipizide in 50 ml of *methanol*, with the aid of ultrasound for 15 minutes with intermittent shaking, and dilute to 100.0 ml with the buffer solution, further sonicate for 15 minutes, filter.

Reference solution. A solution containing 0.01 per cent w/v of *glipizide IPRS* and 0.00005 per cent w/v of *glipizide related compound A* (*N-{2-[(4-aminosulfonyl)phenyl]ethyl}-5-methyl-pyrazinecarboxamide*) *IPRS* in *methanol*. Dilute 25.0 ml of the solution to 50.0 ml with the buffer solution.

Chromatographic system
- a stainless steel column 15 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of the buffer solution and 45 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

The relative retention time with reference to glipizide for glipizide related compound A is about 0.2.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to glipizide related compound A and glipizide is not less than 1.5 and the relative standard deviation for replicate injections is not more than 5.0 per cent for glipizide related compound A.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to glipizide related compound A is not more than four times the area of the corresponding peak in the chromatogram obtained with the reference solution (2.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one intact tablet in 50 ml of *methanol*, with the aid of mechanical shaking for 10 minutes, dilute to 100.0 ml with the buffer solution and further sonicate for 15 minutes. Dilute if necessary with equal volumes of the buffer solution and *methanol* to obtain a solution having similar concentration to the reference solution as obtained in Assay.

Inject the reference solution and the test solution.

Calculate the content of the $C_{21}H_{27}N_5O_4S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 5 mg of Glipizide in 50 ml of *methanol*, with the aid of ultrasound for 15 minutes with intermittent shaking and dilute to 100.0 ml with the buffer solution, further sonicate for 15 minutes and filter.

Reference solution. A 0.01 per cent w/v solution of *glipizide IPRS* in *methanol*. Dilute 25.0 ml of the solution to 50.0 ml with the buffer solution.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{27}N_5O_4S$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Glipizide and Metformin Tablets

Glipizide and Metformin Hydrochloride Tablets

Glipizide and Metformin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of glipizide, $C_{21}H_{27}N_5O_4S$ and metformin hydrochloride, $C_4H_{11}N_5$, HCl.

Usual strengths. Glipizide, 2.5 mg and Metformin Hydrochloride, 250 mg; Glipizide, 5 mg and Metformin Hydrochloride, 250 mg; Glipizide, 5 mg and Metformin Hydrochloride, 500 mg.

Identification

In the Assay of glipizide, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a) and in the Assay of metformin hydrochloride, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

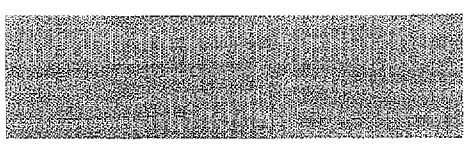
Tests

NOTE — Protect the solution from light.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 1000 ml of 0.05 M phosphate buffer pH 6.8 prepared by dissolving 6.48 g of *monobasic potassium phosphate* and



0.83 g of *sodium hydroxide* in 1000 ml of *water*, adjusted to pH 6.8 with *dilute sodium hydroxide solution*, *(NOTE — Control of the pH is critical)*,

Speed and time. 50 rpm and 45 minutes for glipizide and 30 minutes for Metfonnin hydrochloride.

For Glipizide —

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.005 per cent w/v solution of *glipizide IPRS* in *methanol*. Dilute a suitable volume of the solution with the dissolution medium to obtain the same concentration as expected in the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 48 volumes of a buffer solution prepared by dissolving 3.4 g of *monobasic potassium phosphate* in 800 ml of *water*, adjusted to pH 6.0 with *10 M sodium hydroxide*, dilute to 1000 ml with *water* and 52 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{27}N_5O_4S$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{21}H_{27}N_5O_4S$.

For Metformin Hydrochloride —

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with dissolution medium if necessary, at the maximum at about 233 nm (2.4.7). Calculate the content of $C_4H_{11}N_5$,HCl, in the medium from the absorbance obtained from a solution of known concentration of *metformin hydrochloride IPRS* in the dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_4H_{11}N_5$,HCl.

Related substances. Determine by liquid chromatography (2.4.14).

For Glipizide —

Solvent mixture. 60 volumes of *acetonitrile* and 40 volumes of *water*.

Buffer solution. Dissolve 2.6 g of *dibasic ammonium phosphate* in 1000 ml of *water*, adjusted to pH 8.0 with *ammonium hydroxide*.

Test solution. Disperse 5 intact tablets in the solvent mixture (50.0 per cent of the final volume) in a suitable volumetric flask with the aid of ultrasound for 30 minutes and shake vigorously for another 30 minutes to dissolve and dilute to volume with *water* to obtain a solution containing 0.005 per cent w/v of Glipizide (solution A). To 5.0 ml of solution A, add 5.0 ml of the solvent mixture and dilute to 20.0 ml with *water*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 25 volumes of the buffer solution, 70 volumes of *water* and 5 volumes of *acetonitrile*,
  B. a mixture of 25 volumes of the buffer solution, 25 volumes of *water* and 50 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 223 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
3	100	0
18	0	100
20	0	100
22	100	0
30	100	0

Name	Relative retention time	Correction factor
Glipizide related compound A[1]	0.92	0.71
Glipizide	1.0	—

[1]*N*-{2-[(4-aminosulfonyl)phenyl]ethyl}-5-methyl-pyrazinecarboxamide.

Inject the test solution. The area of any peak corresponding to glipizide related compound A is not more than 2.0 per cent, the area of any other secondary peak (eluting after approximately 8 minutes) is not more than 0.5 per cent and the sum of areas of all the secondary peaks other than glipizide related compound A is not more than 1.0 per cent, calculated by area normalisation. Ignore the peak due to metformin hydrochloride and any peak with an area less than 0.05 per cent.

For Metformin Hydrochloride —

Buffer solution. Dissolve 9.41 g of *sodium 1-hexanesulfonate* in 1000 ml of *water*, adjusted to pH 2.0 with *trifluoroacetic acid*.

Solution B. A mixture of 60 volumes of *water* and 40 volumes of *acetonitrile*.

Solvent mixture. 63 volumes of *water*, 30 volumes of the buffer solution and 7 volumes of *acetonitrile*.

Test solution. Dilute a suitable volume of solution A (obtained from test solution of Glipizide) with the solvent mixture to obtain a solution containing 0.01 per cent w/v of Metformin Hydrochloride.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with phenyl group bonded to porous silica (3.5 µm),
- mobile phase: a mixture of 30 volumes of the buffer solution, 20 volumes of solution B and 50 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume: 25 µl.

Inject the test solution. The area of any secondary peak is not more than 0.1 per cent and the sum of the areas of all the secondary peaks is not more than 0.5 per cent, calculated by area normalization and ignore the peak due to glipizide and any peak with an area less than 0.05 per cent.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Related substances of Glipizide with the following modifications.

Test solution. Disperse 1 intact tablet in the solvent mixture (50.0 per cent of the final volume) in a suitable volumetric flask with the aid of ultrasound for 30 minutes and shake vigorously for another 30 minutes to dissolve and dilute to volume with *water* to obtain a solution containing 0.00125 per cent w/v of Glipizide.

Reference solution. Dissolve 10 mg of *glipizide IPRS* in 60 ml of *acetonitrile*, with the aid of ultrasound for 20 minutes with intermittent shaking and dilute to 100.0 ml with *water*. To 25.0 ml of the solution, add 75 ml of the solvent mixture and dilute to 200.0 ml with *water*.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{27}N_5O_4S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay.

For Glipizide —

Determine by liquid chromatography (2.4.14), as described under Related substances for glipizide with the following modifications.

Reference solution (a). Dissolve 10 mg of *glipizide IPRS* in 60 ml of *acetonitrile*, with the aid of ultrasound for 20 minutes with intermittent shaking and dilute to 100.0 ml with *water*. To 25.0 ml of the solution, add 75 ml of the solvent mixture and dilute to 200.0 ml with *water*.

Reference solution (b). Dissolve 2.5 mg of *glipizide related compound A IPRS* in 150 ml of *acetonitrile*, with the aid of ultrasound for 30 minutes and dilute to 250.0 ml with *acetonitrile*. Dilute 1.0 ml of the solution to 50.0 ml with reference solution (a).

The relative retention time with reference to glipizide for glipizide related compound A is about 0.92.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to glipizide related compound A and glipizide is not less than 1.2 in the chromatogram obtained with reference solution (b) and the relative standard deviation for the replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{21}H_{27}N_5O_4S$ in the tablets.

For Metformin Hydrochloride —

Determine by liquid chromatography (2.4.14), as described under Related substances for metformin hydrochloride with the following modifications.

Reference solution (a). A 0.01 per cent w/v solution of *metformin hydrochloride IPRS* in the solvent mixture.

Reference solution (b). A 0.0005 per cent w/v solution of *dicyandiamide IPRS* in *water*. Dilute 1.0 ml of the solution to 100.0 ml with reference solution (a).

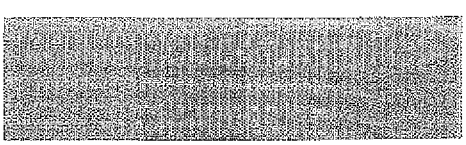
The relative retention time with reference to metformin for dicyandiamide is about 0.26.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to dicyandiamide and metformin is not less than 3.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for the replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

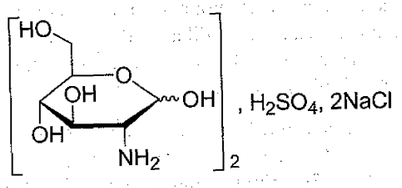
Inject reference solution (a) and the test solution.

Calculate the content of $C_4H_{11}N_5,HCl$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.



Glucosamine Sulphate Sodium Chloride



$C_{12}H_{26}N_2O_{10},H_2SO_4,2NaCl$ Mol. Wt. 573.3

Glucosamine Sulphate Sodium Chloride is bis(2-amino-2-deoxy-*D*-glucopyranose)sulphate bis(sodium chloride).

Glucosamine Sulphate Sodium Chloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{26}N_2O_{10},H_2SO_4,2NaCl$ calculated on the dried basis.

Category. In the treatment of osteoarthritis.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *glucosamine sulphate sodium chloride IPRS* or with the reference spectrum of glucosamine sulphate sodium chloride.

B. It gives reaction (A) of chlorides (2.3.1).

C.1 ml of solution A gives reaction (A) of sodium salts. (2.3.1).

D. It gives reaction (A) of sulphates (2.3.1).

Tests

Solution A. A 10.0 per cent w/v solution in *carbon dioxide-free water*.

Appearance of solution. Dilute 5.0 ml of solution A to 25.0 ml with *water*. The solution is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 3.0 to 5.0 determined in solution A.

Specific optical rotation (2.4.22). +50.0° to +55.0°, determined in solution A at 20°.

(*NOTE — Examine after 3 hour of preparation of solution A*).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.4 g of the substance under examination in 80 ml of the mobile phase with the aid of ultrasound and dilute to 100.0 ml with the mobile phase.

Reference solution. Dissolve 25.0 mg of *2-methylpyrazine IPRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (3µm),
- mobile phase: dissolve 0.5 g of *sodium heptanesulphonate* in *water*, add 0.5 ml of *orthophosphoric acid* and 4 ml of 5.6 per cent w/v solution of *potassium hydroxide* and dilute to 1000.0 ml with *water*. To 1000 ml of the solution add 50 ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 195 nm,
- injection volume: 20 µl.

Inject the reference solution . The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of 2-methylpyrazine.

In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of principal peak in the chromatogram obtained with reference solution (0.05 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (0.2 per cent). Ignore any peak with the area less than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (0.03 per cent).

Heavy metals (2.3.13). Dissolve 2 g of substance under examination in 20 ml of *water*.12 ml of the solution complies with the limit test for heavy metals, Method D (10 ppm), using 10 ml of *lead standard solution (1 ppm Pb)*.

Sulphated ash (2.3.18). 23.5 per cent to 26.0 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

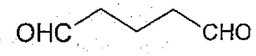
Microbial contamination (2.2.9). Total microbial count not more than 1000 CFU per g, total yeast and mold count not more than 100 CFU per g, 1 g is free from *Escherichia coli*.

Assay. Dissolve 0.25 g in 50 ml of *water* add 1.0 ml of *0.1 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02867 g of $C_{12}H_{28}Cl_2N_2Na_2O_{14}S$.

Storage. Store protected from light.

Glutaraldehyde Solution



$C_5H_8O_2$ Mol. Wt.100.1

Glutaraldehyde Solution is a dilution of Strong Glutaraldehyde Solution in a mixture of purified water and ethanol (95 per cent). For preparation of Glutaraldehyde Solution, ethanol (95 per cent) may be replaced by industrial methylated spirit.

Glutaraldehyde Solution contains not less than 9.2 per cent w/v and not more than 10.5 per cent w/v of glutaraldehyde, $C_5H_8O_2$.

Usual strengths. 0.1 per cent; 1.0 per cent.

Identification

A. Heat 5 ml with 10 ml of a solution containing 1 g of *hydroxylamine hydrochloride* and 2 g of *sodium acetate* on a water-bath for 10 minutes, allow to cool and filter. The melting point (2.4.21) of the residue, after washing with *water* and drying at 105°, is about 178°.

B. To 1 ml, add 2 ml of *ammoniacal silver nitrate solution* and mix gently for a few minutes; silver is deposited.

Tests

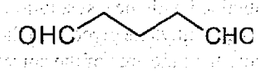
Ethanol (2.3.45). 50.0 to 60.0 per cent v/v.

Assay. Dissolve 4 g with 100 ml of a 7 per cent w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with *1 M sodium hydroxide* and allow to stand for 30 minutes. Add 20 ml of *petroleum spirit (boiling range, 40° to 60°)* and titrate with *1 M sodium hydroxide* until the colour of the aqueous phase matches that of a 7 per cent w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with *1 M sodium hydroxide*.

1 ml of *1 M sodium hydroxide* is equivalent to 0.05005 g of $C_5H_8O_2$.

Labelling. The label states (1) the date after which the solution is not intended to be used; (2) the conditions under which it should be stored.

Strong Glutaraldehyde Solution



$C_5H_8O_2$ Mol. Wt.100.1

Strong Glutaraldehyde Solution is an aqueous solution of glutaraldehyde (pentanedial).

Strong Glutaraldehyde Solution contains not less than 47.0 per cent and not more than 53.0 per cent w/w of glutaraldehyde, $C_5H_8O_2$.

Description. A colourless or almost colourless solution.

Identification

A. Heat 1 ml with 10 ml of a solution containing 1 g of *hydroxylamine hydrochloride* and 2 g of *sodium acetate* in *water* on a water-bath for 10 minutes, allow to cool and filter. The melting point of the residue, after washing with *water* and drying at 105°, is about 178° (2.4.21).

B. Add 0.05 ml to 2 ml of *ammoniacal silver nitrate solution* and mix gently for a few minutes; silver is deposited.

Tests

Acidity. Dilute 10 ml with 10 ml of *carbon dioxide-free water* and titrate with *0.1 M sodium hydroxide* using *bromothymol blue solution* as indicator. Not more than 5.0 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Appearance of solution. Dilute 1 volume with 4 volumes of *water*. The solution is clear (2.4.1) and not more intensely coloured than BS6 (2.4.1).

Weight per ml (2.4.29). 1.126 to 1.134 g.

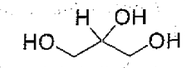
Assay. Dissolve 4 g in 100 ml of a 7 per cent w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with *1 M sodium hydroxide* and allow to stand for 30 minutes. Add 20 ml of *petroleum spirit (boiling range, 40° to 60°)* and titrate with *1 M sodium hydroxide* until the colour of the aqueous phase matches that of a 7 per cent w/v solution of *hydroxylamine hydrochloride* previously neutralised to *bromophenol blue solution* with *1 M sodium hydroxide*.

1 ml of *1 M sodium hydroxide* is equivalent to 0.05005 g of $C_5H_8O_2$.

Storage. Store at a temperature not exceeding 15°.

Glycerin

Glycerol



$C_3H_8O_3$ Mol. Wt. 92.1

Glycerin is propane-1,2,3-triol.

Glycerin contains not less than 98.0 per cent and not more than 101.0 per cent of $C_3H_8O_3$, calculated on the anhydrous basis.

Category. Lubricant; laxative; pharmaceutical aid (humectant).

Description. A clear, colourless or almost colourless, syrupy liquid; hygroscopic.

Identification

Test C may be omitted if tests A and B are carried out. Test A may be omitted if tests B and C are carried out.

A. Mix 5 ml of Glycerin with 1 ml of *water*. On the solution, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glycerin IPRS* treated in the same manner or with the reference spectrum of glycerin.

B. Refractive index (2.4.27). 1.470 to 1.475, determined at 20°.

C. Relative density (2.4.29). 1.258 to 1.268, determined at 20°.

Tests

Appearance of solution. Mix 50.0 g of Glycerin in *carbon dioxide-free water* and dilute to 100.0 ml with the same solvent (Solution A). Solution A is clear (2.4.1). Dilute 10.0 ml of solution A to 25.0 ml with *water*. The solution is colourless (2.4.1).

Acidity or alkalinity. To 50 ml of solution A, add 0.5 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.2 ml of *0.1 M sodium hydroxide* is required to produce a pink colour. Reserve this solution for the test for Esters.

Chlorides (2.3.12). 10.0 ml of solution A, complies with the limit test for chlorides (10 ppm), using 2 ml of chloride standard solution (25 ppm).

Aldehydes. To 7.5 ml of solution A in a glass-stoppered flask, add 7.5 ml of *water* and 1.0 ml of *decolourised pararosaniline solution*, close the flask and allow to stand for 1 hour at 25°. The absorbance of the solution measured at 552 nm (2.4.7) is not more intense than that obtained in a standard solution prepared in the same manner by using 7.5 ml of *formaldehyde standard solution* (5 ppm CH_2O) and 7.5 ml of *water*. The test is not valid unless the standard solution is pink. (10 ppm).

Esters. Add 10 ml *0.1 M sodium hydroxide* to the solution reserved in the test for Acidity or alkalinity. Boil under a reflux condenser for 5 minutes. Cool, add 0.5 ml of *phenolphthalein solution* and titrate with *0.1 M hydrochloric acid*. Not less than 8.0 ml of *0.1 M hydrochloric acid* is required to decolourise the solution.

Halogenated compounds. Not more than 35 ppm.

To 10 ml of solution A, add 1 ml of *dilute sodium hydroxide solution*, 5 ml of *water* and 50 mg of *halogen-free nickel-aluminium alloy*. Heat on a water-bath for 10 minutes, allow to cool and filter. Rinse the flask and the filter with *water* until 25 ml of filtrate is obtained. To 5 ml of the filtrate, add 4 ml of *ethanol (95 per cent)*, 2.5 ml of *water*, 0.5 ml of *nitric acid* and 0.05 ml of *silver nitrate solution* and mix. Allow to stand for 2 minutes. Any opalescence in the solution is not more intense than that in a standard prepared at the same time by mixing 7.0 ml of *chloride standard solution (5 ppm Cl)*, 4 ml of *ethanol (95 per cent)*, 0.5 ml of *water*, 0.5 ml of *nitric acid* and 0.05 ml of *silver nitrate solution*.

Ethylene glycol, diethylene glycol and related substances. Determine by gas chromatography (2.4.13).

Test solution. Mix 5.88 g of Glycerin in *methanol* and dilute to 100.0 ml with *methanol*.

Reference solution (a). A solution containing 0.1 per cent w/v, each of, *ethylene glycol IPRS* and *diethylene glycol IPRS* in *methanol*. To 5.0 ml of the solution, add accurately weighed quantity of 5.88 g of Glycerin and dilute to 100.0 ml with *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v, each of, *glycerin*, *ethylene glycol IPRS* and *diethylene glycol IPRS* in *methanol*.

Chromatographic system

- a fused silica column 30 m × 0.32 mm, packed with 14 per cent cyanopropylphenyl and 86 per centdimethyl-polysiloxane (1µm) (Such as DB-1701),
- temperature:
  column. 100° to 220° @ 7.5° per minute, maintained at 220°,
  inlet port. 220° and detector 250°,
- split ratio: 1:20,
- flame ionization detector,
- flow rate: 38 cm per second using nitrogen as thecarrier gas,
- injection volume: 1µl.

The elution order is ethylene glycol, diethylene glycol and glycerin.

Inject reference solution (a) and (b). Run the chromatogram 3 times the retention time of the glycerin peak. The test is not valid unless the resolution between the peaks due to ethylene glycol and diethylene glycol is not less than 40 and between the peaks due to diethylene glycol and glycerin is not less than 10 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 10 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to ethylene glycol and diethylene glycol, each of, is not more than the area of the corresponding peaks in the chromatogram obtained with reference solution (a) (0.1 per cent) and the area of any other secondary peak is not more than 0.1 per cent, calculated by area normalisation.

The sum of all the impurities is not more than 1.0 per cent.

Sugars. Heat 10 ml of solution A with 1 ml of *dilute sulphuric acid* on a water-bath for 5 minutes. Add 3 ml of *dilute sodium hydroxide* (carbonate-free), mix and add drop wise 1 ml of freshly prepared *copper sulphate solution;* a clear blue solution is produced. Continue heating on the water-bath for 5 minutes; the solution remains blue and no precipitate is produced.

Heavy metals (2.3.13). Mix 4.0 g of Glycerin in 2 ml of *0.1 M hydrochloric acid* and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Sulphated ash (2.3.18). Not more than 0.01 per cent, determined on 5.0 g after heating to boiling and ignition.

Water (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

Assay. Mix 75 mg in 45 ml of *water,* add 25.0 ml of a mixture of 1 volume of *0.1 M sulphuric acid* and 20 volumes of *0.1 M sodium periodate*. Allow to stand protected from light for 15 minutes. Add 5 ml of a 50 per cent w/v solution of *ethylene glycol,* allow to stand protected from light for 20 minutes and titrate with *0.1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.00921 g of $C_3H_8O_3$.

Storage. Store protected from moisture.

Glycerin Oral Solution

Glycerin Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of glycerin, $C_3H_8O_3$.

Category. Soothing agent.

Usual strength. 50 per cent.

Identification

Heat a few drops with 500 mg of *potassium bisulphate* in a test tube; pungent vapors of acrolein are evolved.

Tests

pH (2.4.24). 5.5 to 7.5.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Transfer 3 g of glycerin into a 500-ml volumetric flask, dilute with *water* to volume, and mix. Transfer 3 ml to a conical flask, add 100.0 ml of 0.3 per cent w/v solution of *potassium periodate* in *water*, swirl, and allow to stand at room temperature for 10 minutes. Add 4 g of *sodium bicarbonate* and 2 g of *potassium iodide*. Titrate immediately with *0.1 M potassium arsenite*, using 3 ml of *starch TS* as indicator. Carry out a blank titration.

1 ml of *0.1 M potassium arsenite* is equivalent to 0.002303 g of $C_3H_8O_3$.

Storage. Store protected from moisture.

Glyceryl Monostearate

Monostearin

Glyceryl Monostearate is a mixture of monoglycerides of stearic and palmitic acids, together with variable quantities of di- and triglycerides.

Glyceryl Monostearate contains not less than 35.0 per cent of monoglycerides, calculated as glyceryl monostearopalmitate, $C_{20}H_{40}O_4$, and not more than 7.0 per cent of free glycerin $C_3H_8O_3$, both calculated on the anhydrous basis.

Category. Pharmaceutical aid (emulsifying agent).

Description. A white or almost white, hard, waxy mass or unctuous powder or flakes.

Identification

A. Heat 1 g with 2 g of *potassium bisulphate* in an evaporating dish. Irritant, lachrymatory fumes are evolved which darken filter paper impregnated with *alkaline potassium mercuri-iodide solution.*

B. Heat 2.5 g with 40 ml of *ethanolic potassium hydroxide solution* for 30 minutes on a water-bath under a reflux condenser. Add 30 ml of *water*, evaporate the *ethanol*, acidify the hot mixture with 15 ml of *dilute hydrochloric acid*, cool and extract with 50 ml of *ether*. Wash the *ether* layer with two quantities, each of 10 ml, of a 20 per cent w/v solution of *sodium chloride*, dry the ether extract over *anhydrous sodium sulphate* and filter. Evaporate the solvent and dry the residue under reduced pressure. Melt the residue and fill one or two capillary tubes (for the determination of melting range) and allow to stand for 24 hours in a desiccator. Carry out the determination of melting range by Method II (2.4.21); the residue melts at 54° to 64°.

Tests

Acid value (2.3.23). Not more than 5.0, determined on 0.5 g dissolved in 50 ml of a mixture of equal volumes of *ethanol (95 per cent)* and *ether*.

Saponification value (2.3.37). 155 to 170.

Iodine value (2.3.28). Not more than 5.0 *(iodine bromide method)*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g dissolved in a mixture of 10 ml of *anhydrous methanol* and 10 ml of anhydrous *chloroform*.

Assay. Weigh 0.4 g and dissolve in 50 ml of *dichloromethane* in a glass-stoppered separating funnel. Add 25 ml of *water* and shake vigorously for 1 minute. Allow the layers to separate (if an emulsion is formed, add a few drops of *glacial acetic acid)*. Repeat the extraction with three further quantities, of 25, 20 and 20 ml, of *water* and reserve the dichloromethane solution (solution A). Filter the combined aqueous extracts through a filter paper moistened with *water*, wash the filter with two quantities, each of 5 ml, of *water* and dilute the combined filtrate and washings to 100.0 ml with *water* (solution B).

For monoglycerides — Filter solution A through a cotton wool plug. Wash the separating funnel and the filter with three quantities, each of 5 ml, of *dichloromethane*. Dilute the combined filtrate and washings to 100.0 ml with *dichloromethane*. To 25.0 ml of the solution add 25.0 ml of *periodic-acetic acid solution*, shake cautiously, allow to stand at 25° to 30° for 30 minutes, add 100 ml of *water* and 12 ml of *potassium iodide solution*. Titrate the liberated iodine with *0.1 M sodium thiosulphate* using 1 ml of *starch solution* as indicator. Repeat the determination using 25 ml of *dichloromethane* instead of 25.0 ml of the solution under examination. The difference between the titrations represents the amount of sodium thiosulphate required.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.0172 g of monoglycerides, calculated as glyceryl monostearo-palmitate, $C_{20}H_{40}O_4$.

The quantity of *0.1 M sodium thiosulphate* used in the assay is not less than 85 per cent of the quantity of *sodium thiosulphate* used in the blank assay.

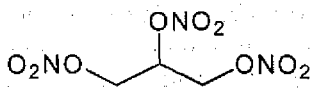
For free glycerin — To 50.0 ml of solution B in a 400-ml conical flask fitted with a ground-glass stopper add 25.0 ml of *periodic-acetic acid solution*, shake cautiously, allow to stand at 25° to 30° for 30 minutes, add 100 ml of *water* and 12 ml of *potassium iodide solution*. Titrate the liberated iodine with *0.1 M sodium thiosulphate* using 1 ml of *starch solution* as indicator. Repeat the determination using 50 ml of *water* instead of 50 ml of the solution under examination. The difference between the titrations represents the amount of *sodium thiosulphate* required.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.0023 g of glycerin, calculated as $C_3H_8O_3$.

Storage. Store protected from light.

Diluted Glyceryl Trinitrate

Diluted Nitroglycerin



$C_3H_5N_3O_9$ Mol. Wt. 227.1

Diluted Glyceryl Trinitrate is 1,2,3-Propanetriol trinitrate.

Diluted Glyceryl Trinitrate is a mixture of nitroglycerin with lactose, dextrose, alcohol, propylene glycol, or other suitable inert excipient to permit safe handling.

Diluted Glyceryl Trinitrate contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of $C_3H_5N_3O_9$. It usually contains not more than 10.0 per cent of glyceryl trinitrate.

CAUTION — Undiluted glyceryl trinitrate can be exploded by percussion or excessive heat. Proper precautions should be exercised in handling it and only exceedingly small amounts should be isolated.

Category. Coronary vasodilator.

Description.A clear, colourless to pale yellow solution or a white powder depending upon the diluent.

Identification

A. In the test of Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to the spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *toluene* and 20 volumes of *ethyl acetate*.

Spray reagent. Dissolve 1g of *diphenylamine* to 100.0 ml with *methanol*.

Test solution (a). Dissolve a quantity of the substance under examination in *methanol* to obtain a solution containing 1.0 per cent w/v of nitroglycerin and centrifuge, if necessary, to obtain a clear liquid solution or apply directly 1.0 per cent w/v nitroglycerin.

Test solution (b). Dilute test solution (a) in *methanol* to obtain 0.04 per cent w/v of nitroglycerin.

Reference solution. Dissolve a quantity of *diluted glyceryl trinitrate IPRS* in *methanol* to obtain 0.04 per cent w/v of nitroglycerin.

Apply to the plate 40 µl each of test solution (a) and (b); 5, 10, 15 and 20 µl of reference solutions. Allow the mobile phase to raise 8 cm. Dry the plate in air and spray the plate with spray reagent and irradiate the plate with short and long wavelength UV light for 15 minutes. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with the reference solution of 20 µl. Compare the intensities of any secondary spots obtained with test solution (a) with those of the principal spots obtain with the reference solution (Corresponding to 0.5 per cent, 1.0 per cent, 1.5 per cent and 2.0 per cent respectively). The sums of the intensities of all the secondary spots are not more than 3.0 per cent. [Note: Nitrates of glycerin typically R_f values of 0.21, 0.37, and 0.61 for mono-, di- and tri-substituted glycerins, respectively]

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the substance under examination equivalent to 7.5 mg of the Glyceryl Trinitrate in 75 ml of mobile phase. If necessary, sonicate for 2 minutes or until the solid is totally dispersed, than shake by mechanical means for 30 minutes and dilute to 100.0 ml with the mobile phase.

Reference solution. Dissolve a quantity of *diluted glyceryl trinitrate IPRS* to obtain 0.0075 per cent w/v of Glyceryl Trinitrate in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_3H_5N_3O_9$.

Storage. Store protected from light at a temperature between15° and 30°.

Glyceryl Trinitrate Tablets

Nitroglycerin Tablets; Trinitrin Tablets

Glyceryl Trinitrate Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of glyceryl trinitrate, $C_3H_5N_3O_9$.

Usual strengths. 300 µg; 500 µg; 600 µg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *toluene*, 20 volumes of *ethyl acetate* and 5 volumes of *glacial acetic acid*.

Test solution. Extract a quantity of the powdered tablets containing 1 mg of glyceryl trinitrate with 1 ml of *acetone* in a glass stoppered vessel; shake for 30 minutes and filter.

Reference solution. Dissolve a quantity of *diluted glyceryl trinitrate IPRS* in *acetone* to obtain 0.1 per cent w/v of nitroglycerin.

Apply to the plate 20 µl of each solution. Allow the mobile phase to raise 8 cm. Dry the plate in air, spray with *diphenylamine solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to peak in the chromatogram obtained with the reference solution.

Tests

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following test solution.

Test solution. Disperse one intact tablet with sufficient mobile phase to produce 0.0075 per cent w/v of nitroglycerin..

Inject reference solution and the test solution.

Calculate the content of $C_3H_5N_3O_9$ in the tablets.

Other tests. Comply with the tests stated under Tablets. The test for Disintegration does not apply.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 7.5 mg of Glyceryl Trinitrate in 75 ml of mobile phase. If necessary, sonicate for 2 minutes or until the solid is totally dispersed, than shake by mechanical means for 30 minutes and dilute to 100.0 ml with the mobile phase.

Reference solution. Dissolve a quantity of *diluted glyceryl trinitrate IPRS* to obtain 0.0075 per cent w/v of Glyceryl Trinitrate in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_3H_5N_3O_9$ in the tablets.

Storage. Store protected from light and moisture in glass containers of not more than 100 tablets, at a temperature not exceeding 30°. The container should be closed by means of a screw cap lined with aluminium or tin foil. Cotton wool wadding or other additional packing that absorbs glyceryl trinitrate should be avoided.

Labelling. The label states that the tablets should be allowed to dissolve slowly in the mouth.

Glycine Irrigation Solution

Glycine Irrigation Solution is a sterile solution of Glycine in Water for Injections.

Glycine Irrigation Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of $C_2H_5NO_2$. It contains no antimicrobial agent.

Usual strength. 1.5 per cent w/v.

Description. A clear, colourless solution.

Identification

A. Evaporate 5 ml to dryness on a water-bath and dry at 105° for one hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glycine IPRS* or with the reference spectrum of glycine.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 70 volumes of *1-propanol* and 30 volumes of *strong ammonia solution*.

Test solution. Dilute a suitable volume of the preparation under examination with *water* so that the resulting solution contains 0.25 per cent w/v of Glycine.

Reference solution. A 0.25 per cent w/v solution of *glycine IPRS*.

Apply to the plate 2 µl of each solution. After development, dry the plate at 105° for 10 minutes, spray with *ninhydrin solution* and heat at 105° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 6.5.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

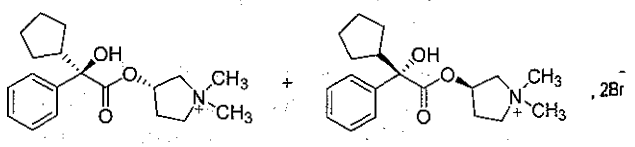
Assay. Dilute a measured volume of the preparation under examination containing about 0.15 g of Glycine to 25 ml with *water*. Add 10 ml of *formaldehyde solution*, previously adjusted to a pH of 9.0, and 0.25 ml of a mixed indicator solution prepared by dissolving 75 mg of *phenolphthalein* and 25 mg of *thymol blue* in 100 ml of *ethanol (50 per cent)*. Titrate with *0.1 M sodium hydroxide* until the yellow colour disappears and a faint violet colour appears.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.007507 g of $C_2H_5NO_2$.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) Not for Injection; (2) that the solution should not be used if it contains visible particles.

Glycopyrrolate



$C_{19}H_{28}BrNO_3$ Mol. Wt. 398.3

Glycopyrrolate is Pyrrolidinium, 3-[(*SR*)-(cyclopentylhydroxyphenylacetyl)oxy]-1,1-dimethyl-,[*IPRS*-]bromide.

Glycopyrrolate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{28}BrNO_3$, calculated on the dried basis.

Category. Anticholinergic.

Description. A white crystalline powder; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *glycopyrrolate IPRS* or with the reference spectrum of glycopyrrolate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives the reactions of bromides (2.3.1).

Tests

Related substances. A. Determine by liquid chromatography (2.4.14).

NOTE — Used when the synthetic route is 5-nitroisopthalic acid may be present.

Buffer solution. A buffer solution prepared by dissolving 1.0 g of *anhydrous sodium sulphate* and 200 mg of *sodium 1-hexanesulphonate monohydrate* in 650 ml of *water*, to this solution add 3.0 ml of *0.5 M sulphuric acid*, and mix,

Solvent mixture. Prepare a solution of 1.0 g of *anhydrous sodium sulphate*, 6.8 g of *monobasic potassium phosphate*, and 200 mg of *sodium 1-hexanesulphonate monohydrate* in 650 ml of *water*. To this solution add 3.0 ml of *0.5M sulphuric acid*, 150 ml of *methanol* and 200 ml of *acetonitrile* and mix. Adjusted to pH 2.8 with *orthophosphoric acid.*

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of *solvent mixture.*

Reference solution. A solution containing 0.00015 per cent w/v each of *glycopyrrolate IPRS, glycopyrrolate related compound A IPRS, glycopyrrolate related compound B IPRS, glycopyrrolate related compound C IPRS* in *solvent mixture.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. 20 volumes of *acetonitrile*, 15 volumes of *methanol* and 65 volumes of buffer solution,
  B. 50 volumes of *acetonitrile*, 15 volumes of *methanol* and 35 volumes of a buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
10	100	0
25	10	90
35	10	90
37	100	0
45	100	0

Name	Relative retention time
Glycopyrrolate related compound A[1]	0.45
Glycopyrrolate	1.00
Glycopyrrolate related compound B[2]	1.14
Glycopyrrolate related compound C[3]	2.68
Individual impurity	—
Total impurity	—

[1]5-Nitrobenzene-1,3-dicarboxylic acid.

[2]1-Methylpyrrolidin-3-yl-2-cyclopentyl-2-hydroxy-2-phenylacetate.

[3]2-Cyclopentyl-2-hydroxy-2-phenylacetic acid.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to glycopyrrolate and glycopyrrolate related compound B is not less than 2.0, the tailing factor is not more than 2.0, the relative standard deviation is not more than 6.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of peaks due to glycopyrrolate related compound A, glycopyrrolate related compound B, related compound C is not more than the area of the peak due to glycopyrrolate in the chromatogram obtained with reference solution (0.15 per cent), the area of any secondary peak is not more than 0.66 times the area of the peak due to glycopyrrolate in the chromatogram obtained with reference solution (0.10 per cent) and the sum of the areas of all the secondary peaks is not more than 3.33 times the area of the glycopyrrolate peak in the chromatogram obtained with reference solution (0.50 per cent).

NOTE — If the Related substances test other than A is used the label states the article complies with Related substances B.

B. Determine by liquid chromatography (2.4.14).

NOTE—Used when the synthetic route is didehydroglycopyrrolate, chloroglycopyrrolate and methyl cyclopentylmandelate may be present.

Buffer solution. A buffer solution prepared by dissolving 3.4 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 2.5 ± 0.2 with *orthophosphoric acid.*

Solvent mixture. Equal volume of *acetonitrile* and *buffer solution.*

Test solution. Dissolve 25 mg of the substance under examination in 50.0 ml of solvent mixture.

Reference solution (a). A solution containing 0.05 per cent w/v of *glycopyrrolate IPRS*, and 0.0001 per cent w/v of each benzoic acid, *glycopyrrolate related compound B IPRS, glycopyrrolate related compound C IPRS, glycopyrrolate related compound I IPRS* and *glycopyrrolate related compound L IPRS* in the solvent mixture.

Reference solution (b). A 0.0001 per cent w/v solution of *glycopyrrolate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 2.1 mm, packed with octadecylsilane bonded to porous silica (1.7 µm),
- column temperature: 45°,
- mobile phase A. buffer solution,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 0.42 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 2.5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
7	39	61
8	39	61
11	15	85
11.5	15	85
12	95	5
15	95	5

Name	Relative retention time	Correction factor
Benzoic Acid	0.82	0.32
Didehydroglycopyrrolate[1]	0.89	1.00
Glycopyrrolate	1.00	—
Glycopyrrolate base[2]	1.06	0.71
Chloroglycopyrrolate[3]	1.22	0.71
Cyclopentylmandelicacid[4]	1.32	0.53
Methyl cyclopentylmandelate[5]	1.52	0.56
Individual impurity	—	1.00
Total impurity	—	—

[1]3-[2-(cyclopent-1-en-1-yl)-2-hydroxy-2-phenylacetoxy]-1,1-dimethylpyrrolidin-1-ium bromide,

[2]1-Methylpyrrolidin-3-yl-2-cyclopentyl-2-hydroxy-2-phenylacetate,

[3](RS)-3-[(SR)-2-(4-chlorophenyl)-2-cyclopentyl-2-hydroxyacetoxy]-1,1-dimethylpyrrolidin-1-ium bromide,

[4]2-Cyclopentyl-2-hydroxy-2-phenylacetic acid,

[5]Methyl 2-cyclopentyl-2-hydroxy-2-phenylacetate.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to glycopyrrolate and glycopyrrolate related compound B is not less than 2.0. the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of peaks due to benzoic acid, didehydroglycopyrrolate, glycopyrrolate related compound B, glycopyrrolate related compound I, related compound C and related compound L is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent) and the sum of the areas of all the secondary peaks is not more than 5.0 times the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Limit of Erythro Isomer. Determine by liquid chromatography (2.4.14).

Buffer solution. A buffer solution prepared by dissolving 2.8 g of *monobasic sodium phosphate* in 1000 ml of *water*, adjusted to pH 6.5 ± 0.05 with *dilute sodium hydroxide solution*.

Test solution. Dissolve 25 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). A solution containing 0.004 per cent w/v of each *glycopyrrolate erythro isomer IPRS* and *glycopyrrolate IPRS* in *mobile phase*.

Reference solution (b). A 0.001 per cent w/v solution of *glycopyrrolate IPRS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with beta cyclodextrin bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *methanol*, 10 volumes of *acetonitrile* and 40 volumes of a buffer solution,
- flow rate: 1 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 10 µl.

Name	Relative retention time
Erythro isomer (*R,R/S,S*-glycopyrrolate)[1]	0.89
Glycopyrrolate	1.00

[1](*RS*)-3-[(*RS*)-2-cyclopentyl-2-hydroxy-2-phenylacetoxy]-1,1-dimethylpyrrolidinium bromide.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to erythro isomer and glycopyrrolate is not less than 1.2.

Inject reference solution (b). The tailing factor is not more than 2.0. and the relative standard deviation for replicate injections is not more than 6.0 per cent

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of impurity due to erythro isomer (*R,R/S,S*-glycopyrrolate) is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *glycopyrrolate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1.0 g of *anhydrous sodium sulphate* and 200 mg of *sodium 1-hexanesuphonate monohydrate* in 650 ml of *water*; to this solution add 3.0 ml of *0.5 M sulphuric acid*, 20 volumes of *acetonitrile* and 15 volumes of *methanol*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{28}BrNO_3$.

Storage. Store protected from moisture.

Glycopyrrolate Injection

Glycopyrrolate Injection is a sterile solution of Glycopyrrolate in Water for Injection.

Glycopyrrolate Injection contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of glycopyrrolate, $C_{19}H_{28}BrNO_3$.

Usual strengths. 0.2 mg per ml; 2 mg per 10 ml.

Identification

Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 30 volumes of *butyl alcohol*, 10 volumes of *glacial acetic acid* and 10 volumes of *water*.

Spray reagent. A solution prepared immediately by dissolving 2 g of *bismuth subnitrate* in a mixture of 100.0 ml of *water*, and 25.0 ml with *glacial acetic acid* (solution A). Dissolve 40 g of *potassium iodide* in 100.0 ml of *water* (solution B). Mix 10 ml of *(solution A)* and 10 ml of *(solution B)* and add to a solution containing 100 ml of *water* and 20 ml of *glacial acetic acid*.

Test solution. Dilute a volume of injection to obtain a solution containing 0.01 per cent w/v of glycopyrrolate in *water*.

Reference solution. A 0.01 per cent w/v solution of *glycopyrrolate IPRS* in *water*.

Apply to the plate 30 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with reagent. The R_f values and colour of the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 2.0 to 3.0.

Bacterial Endotoxins (2.2.3). Not more than 555.5 Endotoxin Units per mg of glycopyrrolate.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing 5 mg of Glycopyrrolate to 25.0 ml with *mobile phase*.

Reference solution (a). A solution containing 0.02 per cent w/v of *glycopyrrolate IPRS* in *mobile phase*.

Reference solution (b). A 0.05 per cent w/v solution of *benzaldehyde* in *mobile phase*. Dilute 2.0 ml of the solution to 25 ml in reference solution (a).

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A buffer solution prepared by dissolving 1.0 g of *anhydrous sodium sulphate* and 200 mg of *sodium pentanesulphonate monohydrate* in 615 ml of *water*; add 3.0 ml of *0.5 M sulphuric acid*, 235 ml of *acetonitrile* and 150 ml of *methanol* in a 1000-ml volumetric flask,
- flow rate: 2 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 35 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to benzaldehyde and glycopyrrolate is not less than 3.0.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution .

Calculate the content of $C_{19}H_{28}BrNO_3$ in the injection.

Storage. Store in single dose or multi dose containers, preferably of Type I glass.

Glycopyrrolate Tablets

Glycopyrrolate Tablets contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of glycopyrrolate, $C_{19}H_{28}BrNO_3$.

Usual strength. 1 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 500 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate, store the solution at 5°.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. Dissolve a sufficient quantity of *glycopyrrolate IPRS* in minimum quantity of *methanol*, dilute with the dissolution medium to obtain a solution having the concentration similar to the expected concentration of the test solution. Store the solution at 5°.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- sample temperature: 5°,
- mobile phase: a mixture of 620 volumes of a buffer solution prepared by dissolving 1.0 g of *anhydrous sodium sulphate* and 200 mg of *sodium 1-pentanesulphonate* in 620 ml of *water*, 200 volumes of *acetonitrile*, 180 volumes of *methanol* and 3 volumes of *0.5M sulphuric acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 80 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Q. Not less than 75 per cent of the stated amount of $C_{19}H_{28}BrNO_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. A buffer solution prepared by dissolving 1.0 g of *anhydrous sodium sulphate* and 200 mg of *sodium 1-hexanesulphonate monohydrate* in 650 ml of *water*, to this solution add 3.0 ml of *0.5 M sulphuric acid*, and mix.

Solvent mixture. Dissolve 1.0 g of *anhydrous sodium sulphate*, 6.8 g of *monobasic potassium phosphate*, and 200 mg of *sodium 1-hexanesulfonate monohydrate* in 650 ml of *water*. To this solution add 3.0 ml of *0.5M sulphuric acid*, 150 ml of *methanol* and 200 ml of *acetonitrile* and mix. Adjust the pH to 2.8 with *ortho phosphoric acid*.

Test solution. Shake a quantity of the powdered tablets containing 12.5 mg of Glycopyrrolate with 15 ml of the solvent mixture and disperse with the aid of ultrasound for 10 minutes. Shake further, if necessary, dilute to 25.0 ml with solvent mixture and filter through suitable filter, rejecting the first few ml of filtrate.

Reference solution. A solution containing 0.00015 per cent w/v each of *glycopyrrolate IPRS*, *glycopyrrolate related compound B IPRS* and *glycopyrrolate related compound C IPRS* in *solvent mixture*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. 20 volumes of *acetonitrile*, 15 volumes of *methanol* and 65 volumes of the buffer solution,
  B. 50 volumes of *acetonitrile*, 15 volumes of *methanol* and 35 volumes of the buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
10	100	0
25	10	90
35	10	90
37	100	0
45	100	0

Name	Relative retention time
Glycopyrrolate related compound A[1]	0.45
Glycopyrrolate	1.00
Glycopyrrolate related compound B[2]	1.14
Glycopyrrolate related compound C[3]	2.68
Individual impurity	—
Total impurity	—

[5-Nitrobenzene-1,3-dicarboxylic acid,

[1-Methylpyrrolidin-3-yl-2-cyclopentyl-2-hydroxy-2-phenylacetate,

[2-Cyclopentyl-2-hydroxy-2-phenylacetic acid.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to glycopyrrolate and glycopyrrolate related compound B is not less than 2.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 6.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of impurity due to glycopyrrolate related compound C is not more than 1.7 times the area of the principal peak in the chromatogram obtained with reference solution (0.5 per cent), the area of any secondary peak is not more than 0.67 times the area of the principal peak in the chromatogram obtained with reference solution (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 4.0 times the area of the peak in the chromatogram obtained with reference solution (1.2 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in 5.0 ml of the mobile phase, with the aid of ultrasound for 10 minutes and dilute to 10.0 ml with the mobile phase. Centrifuge and use the supernatant liquid. Dilute further, if necessary, with the mobile phase.

Calculate the content of $C_{19}H_{28}BrNO_3$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 10 mg of Glycopyrrolate add 75 ml of mobile phase and disperse by shaking for 30 minutes and mixing if necessary with the aid of ultrasound for further 10 minutes. Dilute to 100.0 ml with the mobile phase, centrifuge and use the filtrate.

Reference solution. A 0.01 per cent w/v solution of *glycopyrrolate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1.0 g of *anhydrous sodium sulphate* and 200 mg of *sodium 1-hexanesuphonate monohydrate* in 650 ml of *water*, to this solution add 3.0 ml of *0.5 M sulphuric acid*, 20 volumes of *acetonitrile* and 15 volumes of *methanol*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 222 nm,
- injection volume: 50 µl.

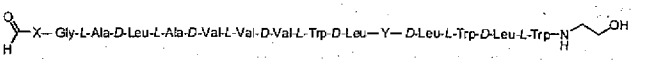
Inject the reference solution.The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{28}BrNO_3$ in the tablets.

Storage. Store protected from moisture.

Gramicidin



Gramicidin	X	Y	Mol. Formula	Mol. Wt.
A1	*L*-Val	*L*-Trp	$C_{99}H_{140}N_{20}O_{17}$	1882
A2	*L*-Ile	*L*-Trp	$C_{100}H_{142}N_{20}O_{17}$	1896
B1	*L*-Val	*L*-Phe	$C_{97}H_{139}N_{19}O_{17}$	1843
C1	*L*-Val	*L*-Tyr	$C_{97}H_{139}N_{19}O_{18}$	1859
C2	*L*-Ile	*L*-Tyr	$C_{98}H_{141}N_{19}O_{18}$	1873

Category. Polypeptide Antibacterial.

Gramicidin consists of a family of antimicrobial linear polypeptides substance obtained by extraction from tyrothricin and produced by fermentation broth of *Brevibacillus brevis* Dubos. The main component is gramicidin A1, together with gramicidins A2, B1, C1 and C2 in particular.

Gramacidin has potency not less than 900 Units per mg, calculated on the dried basis.

Description. A white or almost white crystalline powder; slightly hygroscopic.

Identification

Test B may be omitted if tests A and C are carried out. Tests C may be omitted if test A and B is carried out.

A. Dissolve 0.10 g in *ethanol* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with *ethanol*. When examined in the range 240 nm to 320 nm (2.4.7), the resulting solution shows absorption maxima, at about 282 nm and about 290 nm, a shoulder at about 275 nm and an absorption minimum at about 247 nm. The specific absorbance at the maximum at about 282 nm is 105 to 125.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 3 volumes of *methanol,* 9 volumes of *butanol,* 15 volumes of *water,* 24 volumes of *glacial acetic acid* and 49 volumes of *butyl acetate.*

Test solution. Dissolve 5 mg of the substance under examination in 6.0 ml of *ethanol.*

Reference solution (a). Dissolve 5 mg of *gramicidin IPRS* in 6.0 ml of *ethanol.*

Reference solution (b). Dissolve 5 mg of *tyrothricin IPRS* in 6.0 ml of *ethanol.*

Apply to the plate 1 µl of each solution. After development, dry the plate in air, spray with *dimethylaminobenzaldehyde solution* and heat at 90° until the spots appear. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatograms obtained with reference solution (a) and (b).

C. In the test for Composition, the retention time of three principal peak in the chromatogram obtained with test solution corresponds to the three peak in the chromatogram obtained with reference solution (a).

Tests

Composition. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of *methanol* and dilute to 25.0 ml with mobile phase.

Reference solution (a). Dissolve 25 mg of *gramicidin IPRS* in 10 ml of *methanol* and dilute to 25.0 ml with mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 50.0 ml with mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base dectivated end capped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 71 volumes of *methanol* and 29 volumes of *water.*
- flow rate: 1 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume: 20 µl.

Name	Relative retention time
Gramicidin impurity C1[1]	0.7
Gramicidin impurity C2[2]	0.8
Gramicidin A1(Retention time: about 22 minutes)	1.0
Gramicidin impurity A2[3]	1.2
Gramicidin impurity B1[4]	1.9

[1][10-methionine]gramicidin C1.

[2]gramicidin C2

[3]gramicidin A2 3-hydroxypropyl.

[4]gramicidin B1.

Inject reference solution (a). Run the chromatogram 2.5 times the retention time of gramicidin A1. The test is not valid unless the resolution between the peak due to gramicidin A1 and gramicidin A2 is not less than 1.5.

Inject reference solution (b) and the test solution. The ratio of the content of gramicidin A1 to the sum of the contents of gramicidins A1, A2, B1, C1 and C2 is not less than 60.0 per cent and sum of the contents of gramicidins A1, A2, B1, C1 and C2 is not less than 95.0 per cent. Ignore any peak with area due to gramicidin A1 in the chromatogram obtained with reference solution (b).

Related substances. Determine by liquid chromatography (2.4.14) as described under Composition.

The area peaks other than principal peak in the chromatogram obtained with test solution is not more than 2.0 per cent and not more than 1 peak is more than 1.0 per cent. Ignore any peak with area due to gramicidin A1, A2, B1, C1 and C2.

Melting point (2.4.21). About 230°.

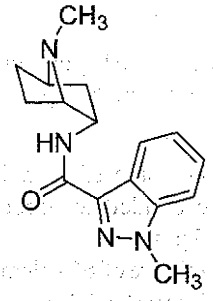
Sulphated ash (2.3.18). Not more than 1.0 per cent, determined on 1.0 g.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 60° over *diphosphorus pentoxide* at a pressure not exceeding 0.1 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method B (2.2.10), and express the result in Units of gramicidin per mg.

Storage. Store protected from light and moisture.

Granisetron



$C_{18}H_{24}N_4O$ Mol. Wt. 312.4

Granisetron is 1-Methyl-*N*-(9-methyl-endo-9-azabicyclo[3.3.1] non-3-yl]-1*H*-indazole-3-carboxamide.

Granisetron contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{24}N_4O$, calculated on the dried basis.

Category. As Antiemetic in cytotoxic drug induced nausea and vomiting.

Description. An off white to pale yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *granisetron IPRS* or with the reference spectrum of granisetron.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Impurity E. Not more than 0.15 per cent. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Solvent mixture. 20 volumes of *water* and 80 volumes of *acetonitrile.*

Mobile phase. 6.5 volumes of *ammonium hydroxide,* 30 volumes of *2-propanol* and 50 volumes of *ethyl acetate.*

Test solution. Dissolve 0.5 g of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution. A 0.0075 per cent w/v solution of *granisetron impurity E IPRS* in the solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose it to iodine vapours for 30 minutes. Any secondary spot, corresponding to the spot obtained with the reference solution, in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Related substances. Determine by liquid chromatography (2.4.14).

(*NOTE — Protect the solutions from light and use the solutions within 4 hours.*)

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the same solvent.

Reference solution (a). A 0.0001 per cent w/v solution of *granisetron IPRS* in the mobile phase.

Reference solution (b). Transfer 2 ml of the test solution to a colourless glass vial, stopper it and expose the solution either to sunlight for 4 hours or under a UV lamp for 16 hours (partial degradation of granisetron to generate impurity C). A degradation of at least about 0.3 per cent of granisetron to impurity C must be obtained as shown by appearance of a corresponding peak in the chromatogram. If not, expose the solution once again to sunlight or under a UV lamp.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 80 volumes of *water,* 20 volumes of *acetonitrile,* 0.1 volume of *hexylamine* and 0.16 volume of *orthophosphoric acid.* Adjusted to pH 7.5 ± 0.05 with *triethylamine,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume: 10 µl.

Name	Relative retention time
Granisetron impurity D[4]	0.4
Granisetron impurity B[2]	0.5
Granisetron impurity A[1]	0.7
Granisetron impurity C[3]	0.8
Granisetron	1.0

[1] 2-methyl-*N*-[(1*R*,3r,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide,

[2] *N*-[(1*R*,3r,5*S*)-9-methyl-9-azabicyclo[3.3.1]nons-3-yl]-1*H*-indazole-3-carboxamide,

[3] *N*-[(1*R*,3r,5*S*)-9-azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide,

[4] 1-methyl-1*H*-indazole-3-carboxylic acid.

Run the chromatogram twice the retention time of peak due to granisetron.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to impurity C and granisetron is not less than 3.5 and the tailing factor is not more than 2.0 in the chromatogram obtained with reference solution (b). The relative standard deviation for replicate injections is not more than 5.0 per cent using granisetron peak in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to granisetron impurity A and granisetron impurity B is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent). The area of any peak due to granisetron impurity C is not more than two times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of any peak due to granisetron impurity D is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained

with reference solution (a) (0.1 per cent). The sum of areas of all secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

Reference solution (a). A 0.1 per cent w/v solution of *granisetron IPRS* in the mobile phase.

Reference solution (b). Transfer 2 ml of the test solution to a colourless glass vial, stopper it and expose the solution either to sunlight for 4 hour or under a UV lamp for 16 hour (partial degradation of granisetron to impurity C). A degradation of at least about 0.3 per cent of granisetron to impurity C must be obtained as shown by appearance of a corresponding peak in the chromatogram. If not, expose the solution once again to sunlight or under a UV lamp.

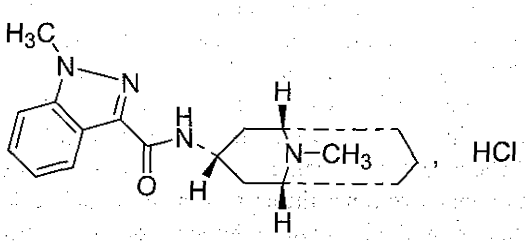
Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to impurity C and granisetron is not less than 3.5 and the tailing factor is not more than 2.0 in the chromatogram obtained with reference solution (b). The relative standard deviation for replicate injections is not more than 0.73 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{24}N_4O$.

Storage. Store protected from light and moisture.

Granisetron Hydrochloride



$C_{18}H_{25}ClN_4O$ Mol. Wt. 348.9

Granisetron Hydrochloride is 1-Methyl-*N*-[(1*R*,3*r*,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide hydrochloride.

Granisetron Hydrochloride contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{18}H_{25}ClN_4O$, calculated on the dried basis.

Category. As Antiemetic in cytotoxic drug induced nausea and vomiting.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *granisetron hydrochloride IPRS* or with the reference spectrum of granisetron hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. A 1.0 per cent w/v solution gives the reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.5, determined in a 1.0 per cent w/v solution.

Impurity E. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel* GF_{254}.

Solvent mixture. 20 volumes of *water* and 80 volumes of *acetonitrile.*

Mobile phase. A mixture of 6.5 volumes of *ammonia*, 30 volumes of *2-propanol* and 50 volumes of *ethyl acetate.*

Test solution. Dissolve 0.5 g of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution. A 0.025 per cent w/v solution of *granisetron impurity E IPRS [(1R,3r,5S)-9-methyl-9-azabicyclo(3.3.1)nonan-3-amine)]* in the solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and expose it to iodine vapours for 30 minutes. Any secondary spot, corresponding to the spot obtained with reference solution, in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect all the solutions from light.

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the same solvent.

Reference solution (a). A 0.1 per cent w/v solution of *granisetron hydrochloride IPRS* in the mobile phase.

Reference solution (b). Transfer 2 ml of the test solution to a colourless glass vial, stopper it and expose the solution either to sunlight for 4 hours or under a UV lamp for 16 hours (partial degradation of granisetron to generate impurity C). A degradation of at least about 0.3 per cent of granisetron to impurity C must be obtained as shown by appearance of a corresponding peak in the chromatogram. If not, expose the solution once again to sunlight or under a UV lamp.

Reference solution (c). Dilute 0.5 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (d). A solution containing 0.001 per cent w/v of *granisetron impurity A IPRS* and 0.0005 per cent w/v of *granisetron impurity B IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 80 volumes of *water*, 20 volumes of *acetonitrile*, 0.1 volume of *hexylamine* and 0.16 volume of *orthophosphoric acid*. Adjusted to pH 7.5 ± 0.05 with *triethylamine*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Granisetron impurity D[4]	0.4	---
Granisetron impurity B[2]	0.5	1.7
Granisetron impurity A[1]	0.7	---
Granisetron impurity C[3]	0.8	---
Granisetron	1.0	---

[1] 2-methyl-*N*-[(1R,3r,5S)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide,

[2] *N*-[(1R,3r,5S)-9-methyl-9-azabicyclo[3.3.1]nons-3-yl]-1*H*-indazole-3-carboxamide,

[3] *N*-[(1R,3r,5S)-9-azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide,

[4] 1-methyl-1*H*-indazole-3-carboxylic acid,

Run the chromatogram twice the retention time of peak due to granisetron.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to impurity C and granisetron is not less than 3.5 and the tailing factor is not more than 2.0 for granisetron peak. The relative standard deviation for replicate injections is not more than 10.0 per cent for granisetron peak.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent). The area of any peak corresponding to impurity C is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent). The area of any peak corresponding to impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). The area of any peak corresponding to impurity D is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The sum of areas of all secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

Inject reference solution (a). The test is not valid unless the relative standard deviation is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{25}ClN_4O$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Granisetron Injection

Granisetron Hydrochloride Injection

Granisetron Injection is a sterile solution of Granisetron Hydrochloride in Water for Injections. It may contain suitable preservatives.

Granisetron Injection contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of granisetron, $C_{18}H_{24}N_4O$.

Usual strength. 1 mg per ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *methylene chloride*, 40 volumes of *alcohol*, 5 volumes of *water* and 2 volumes of *ammonium hydroxide*.

Test solution. Use the undiluted Injection.

Reference solution. Dissolve a quantity of *granisetron hydrochloride IPRS* in *water* to get similar concentration of the test solution.

Apply to the plate 20 µl each of the reference solution and the test solution. Allow the mobile phase to rise 15 cm. Dry the plate under a current of warm air for about 5 minutes and examine the plate under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 4.0 to 6.0.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Perform the determination under subdued light and use amber autosampler vials and low-actinic glassware.

Buffer solution. Dissolve 15.6 g of *monobasic sodium phosphate dihydrate* in 900 ml of *water*, adjusted to pH 2.0 with *orthophosphoric acid* and dilute with *water* to 1000 ml.

Test solution. Dilute the sample with *water* if necessary to obtain a solution having a known concentration of 0.1 per cent w/v of granisetron.

Reference solution (a). A 0.011 per cent w/v solution of *granisetron hydrochloride IPRS* in *water*.

Reference solution (b). A solution containing 0.0001 per cent w/v of each granisetron hydrochloride, granisetron impurity B, C and D in a mixture of 75 volumes of *water* and 25 volumes of *methanol*.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, polar end-capped packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 75 volumes of buffer solution, 24 volumes of *methanol* and 1.1 volume of *tetrahydrofuran*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 15 µl.

Name	Relative retention time	Correction factor
Granisetron impurity A[1]	0.5 to 0.6	—
Granisetron impurity B[2]	0.7	1.25
Granisetron	1.0	—
Granisetron impurity C[3]	1.2	1.0
Granisetron impurity D[4]	2.1 to 2.3	0.67

[1]2-methyl-*N*-[(1*R*,3r,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide,

[2]*N*-[(1*R*,3r,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide,

[3]*N*-[(1*R*,3r,5*S*)-9-azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide,

[4]1-methyl-1*H*-indazole-3-carboxylic acid.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to impurity C and granisetron is not less than 2.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to impurity C is not more than 0.07 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent). The sum of areas of all known impurities peaks is not more than 0.13 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.3 per cent). The area of any other secondary peak is not more than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with relative retention time of about 0.5 to 0.6 for granisetron impurity A. Ignore any peak with an area less than 0.01 times the area of the principal peak obtained in reference solution (a) (0.1 per cent).

Bacterial endotoxins (2.2.3). Not more than 25 Endotoxin unit per mg of granisetron.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

Test solution. A 0.011 per cent w/v solution of Granisetron Hydrochloride in *water*.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{24}N_4O$ in the injection.

Storage. Store protected from light, at temperature not exceeding 30°.

Labelling. The label states (1) the strength in terms of the equivalent amount of granisetron in a suitable dose-volume; (2) the route of injection.

Granisetron Tablets

Granisetron Hydrochloride Tablets

Granisetron Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of granisetron, $C_{18}H_{24}N_4O$.

Usual strength. 1 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *methylene chloride*, 40 volumes of *alcohol*, 5 volumes of *water* and 2 volumes of *ammonium hydroxide*.

Test solution. Disperse a quantity of the powdered tablets containing 4 mg of Granisetron with 10 ml of *0.1 M hydrochloric acid* with the aid of ultrasound for 3 minutes and filter.

Reference solution. A 0.044 per cent w/v solution of *granisetron hydrochloride IPRS* in *0.1 M hydrochloric acid*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 500 ml of a phosphate buffer pH 6.5, prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 800 ml of *water*, adjusted to pH 6.5 with *1 M sodium hydroxide* and diluting to 1000 ml with *water*.

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

NOTE—Perform the determination under subdued light and use amber autosampler vials and low-actinic glassware.

Buffer solution. Dissolve 15.6 g of *monobasic sodium phosphate dihydrate* in 900 ml of *water*, adjust to pH 2.0 with *orthophosphoric acid*, and dilute with *water* to 1000 ml.

Test solution. Use the filtrate, dilute if necessary, with the buffer solution.

Reference solution. Dissolve a quantity of *granisetron hydrochloride IPRS* in the buffer solution to obtain a solution of known concentration similar to the test solution.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, polar end-capped packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 75 volumes of buffer solution, 24 volumes of *methanol* and 1.1 volume of *tetrahydrofuran*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 100 µl.

Inject the reference solution and the test solution.

Q. Not less than 75 per cent of the stated amount of $C_{18}H_{24}N_4O$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Perform the determination under subdued light and use amber autosampler vials and low-actinic glassware.

Buffer solution. Dissolve 15.6 g of *monobasic sodium phosphate dihydrate* in 900 ml of *water*, adjust to pH 2.0 with *orthophosphoric acid*, and dilute with *water* to 1000 ml.

Test solution. Weigh and powder 20 tablets. Transfer a quantity of powder containing 10 mg of granisetron in 100-ml volumetric flask, add about 50 ml of buffer solution and disperse with the aid of ultrasound for about 10 minutes, cool and dilute to volume with buffer solution and filter.

Reference solution (a). A 0.011 per cent w/v solution of *granisetron hydrochloride IPRS* in the buffer solution.

Reference solution (b). A solution containing 0.01 per cent w/v of *granisetron hydrochloride IPRS* and 0.001 per cent w/v each of granisetron impurity B, C and D in the buffer solution.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, polar end-capped packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 75 volumes of buffer solution, 24 volumes of *methanol* and 1.1 volume of *tetrahydrofuran*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Granisetron impurity A[1]	0.5 to 0.6	—
Granisetron impurity B[2]	0.7	1.25
Granisetron	1.0	—
Granisetron impurity C[3]	1.2	1.0
Granisetron impurity D[4]	2.1 to 2.3	0.67

[1]2-methyl-*N*-[(1*R*,3r,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2*H*-indazole-3-carboxamide,

[2]*N*-[(1*R*,3r,5*S*)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-1*H*-indazole-3-carboxamide,

[3]*N*-[(1*R*,3r,5*S*)-9-azabicyclo[3.3.1]non-3-yl]-1-methyl-1*H*-indazole-3-carboxamide,

[4]1-methyl-1*H*-indazole-3-carboxylic acid,

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to impurity C and granisetron is not less than 2, the tailing factor is not less than 0.8 and not more than 1.5 for granisetron peak.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution. The area of any peak corresponding to impurity C is not more than 0.7 per cent. The sum of areas of all the known impurities peaks is not more than 1.3 per cent. The area of any other secondary peak is not more than 0.5 per cent. Ignore any peak with relative retention time about 0.5 to 0.6 for granisetron impurity A. Ignore any peak with an area less than 0.1 per cent. Calculated by area normalisation method.

Uniformity of content. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

Test solution. Disperse 1 tablet in the buffer solution with the aid of ultrasound, and dilute to obtain a solution containing 0.01 per cent w/v solution of Granisetron.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{24}N_4O$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modification.

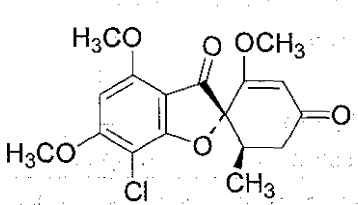
Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{24}N_4O$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of Granisetron.

Griseofulvin



$C_{17}H_{17}ClO_6$ Mol. Wt. 352.8

Griseofulvin is (1'*S*,6'*R*)-7-chloro-2',4,6-trimethoxy-6'-methyl-3*H*-spiro[1-benzofuran-2,1'-cyclohex[2]ene]-3,4'-dione produced by the growth of certain strains of *Penicillium griseofulvum*.

Griseofulvin contains not less than 94.0 per cent and not more than 102.0 per cent of $C_{17}H_{17}ClO_6$, calculated on the dried basis.

Category. Antifungal.

Description. A white to yellowish white powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *griseofulvin IPRS* or with the reference spectrum of griseofulvin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Melting range (2.4.21). 217° to 224°.

Tests

Appearance of solution. A 7.5 per cent w/v solution in *dimethylformamide* is clear (2.4.1), and not more intensely coloured than reference solution YS4 (2.4.1).

Acidity. Suspend 0.25 g in 20 ml of *ethanol (95 per cent)* and titrate with *0.2 M sodium hydroxide* using *phenolphthalein solution* as indicator; not more than 1.0 ml is required to change the colour of the solution.

Specific optical rotation (2.4.22). +354° to +364°, determined at 20° in 1.0 per cent w/v solution in *dimethylformamide*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in mobile phase B and dilute to 50.0 ml with the mobile phase B.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase B.

Reference solution (b). Dissolve 5 mg of *griseofulvin for system suitability IPRS* (containing impurities A, B and C) in 10 ml of mobile phase B.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 20 volumes of 0.1 per cent *v/v* solution of *anhydrous formic acid*, adjusted to pH 4.5 with *dilute ammonia* and 80 volumes of *water*,
  B. a mixture of 15 volumes of *water*, 20 volumes of 0.1 per cent *v/v* solution of *formic acid*,

adjusted to pH 4.5 with *dilute ammonia* and 65 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	50	50
3	50	50
13	40	60
16	10	90
24	10	90
28	50	50

Name	Relative retention time	Correction factor
Griseofulvin impurityA[1]	0.4	0.6
Griseofulvin impurity B[2]	0.7	—
Griseofulvin (Retention time: about 16 minutes)	1.0	—
Griseofulvin impurity C[3]	1.1	—

[1](1*S*,6*R*)-7-chloro-4,6-dimethoxy-6-methyl-3*H*-spiro[1-benzofuran-2,1-cyclohexane]-2,3,4-trione,

[2](1*S*,6*R*)-2,4,6-trimethoxy-6-methyl-3*H*-spiro[1-benzofuran-2,1-cyclohex[2]ene]-3,4-dione,

[3](1*S*)-7-chloro-2,4,6-trimethoxy-6-methyl-3*H*-spiro[1-benzofuran-2,1-cyclohexan[2,5]diene]-3,4-dione.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 3.0, where H_p is the height above the baseline of the peak due to impurity C and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to griseofulvin.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to griseofulvin impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent), the area of any peak corresponding to griseofulvin impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent), the area of any peak corresponding to griseofulvin impurity C is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent), the area of any other secondary peak is not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in mobile phase B and dilute to 50.0 ml with mobile phase B.

Reference solution. A 0.05 per cent w/v solution of *griseofulvin IPRS* in mobile phase B.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{17}ClO_6$.

Storage. Store protected from moisture.

Griseofulvin Tablets

Griseofulvin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of griseofulvin, $C_{17}H_{17}ClO_6$.

Usual strengths. 125 mg; 500 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.125 g of Griseofulvin with 20 ml of *dichloromethane*, add 1 g of *anhydrous sodium sulphate*, shake and filter. Evaporate the filtrate to dryness and dry at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *griseofulvin IPRS* or with the reference spectrum of griseofulvin.

B. Disperse 5 mg of the powdered tablets in 1 ml of *sulphuric acid* and add 5 mg of powdered, *potassium dichromate*; a wine-red colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 1000 ml of a 1.5 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measurethe absorbance of filtrate, suitably diluted with *methanol(80 per cent)*, at the maximum at 291 nm (2.4.7).Calculate the content of $C_{17}H_{17}ClO_6$, taking 725 as the specific absorbance at the maximum at 291 nm.

Q. Not less than 75 per cent of the stated amount of $C_{17}H_{17}ClO_6$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 0.25 g of Griseofulvin in mobile phase B and dilute to 500.0 ml with mobile phase B.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase B.

Reference solution (b). A 0.05 per cent w/v solution of *griseofulvin for system suitability IPRS* in mobile phase B.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (5 µm) (Such as Discovery C18),
- mobile phase: A. a mixture of 20 volumes of 0.1 per cent *v/v* solution of *formic acid*, adjusted to pH 4.5 with *dilute ammonia* and 80 volumes of *water*,
  B. a mixture of 15 volumes of *water*, 20 volumes of 0.1 per cent *v/v* solution of *formic acid*, adjusted to pH 4.5 with *dilute ammonia* and 65 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	50	50
3	50	50
13	40	60
16	10	90
24	10	90
28	50	50

Name	Relative retention time	Correction factor
Griseofulvin impurityA[1]	0.4	0.6
Griseofulvin impurity B[2]	0.7	—
Griseofulvin (Retention time: about 16 minutes)	1.0	—
Griseofulvin impurity C[3]	1.1	—

[1](1*S*,6*R*)-7-chloro-4,6-dimethoxy-6-methyl-3*H*-spiro[1-benzofuran-2,1-cyclohexane]-2,3,4-trione,

[2](1*S*,6*R*)-2,4,6-trimethoxy-6-methyl-3*H*-spiro[1-benzofuran-2,1-cyclohex[2]ene]-3,4-dione,

[3](1*S*)-7-chloro-2,4,6-trimethoxy-6-methyl-3*H*-spiro[1-benzofuran-2,1-cyclohexan[2,5]diene]-3,4-dione.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 3.0, where H_p is the height above the baseline of the peak due to impurity C and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to griseofulvin.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to griseofulvin impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent), the area of any peak corresponding to griseofulvin impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent), the area of any peak corresponding to griseofulvin impurity C is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (5.0 per cent). Ignore any peak with an area less than 0.1 times the area of principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.25 g of Griseofulvin in mobile phase B and dilute to 500.0 ml with mobile phase B.

Reference solution. A 0.05 per cent w/v solution of *griseofulvin IPRS* in mobile phase B.

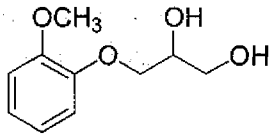
Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{17}ClO_6$ in the tablets.

Guaiphenesin



$C_{10}H_{14}O_4$ Mol. Wt. 198.2

Guaiphenesin is (*RS*)-3-(2-methoxyphenoxy)propane-1,2-diol.

Guaiphenesin contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{10}H_{14}O_4$, calculated on the dried basis.

Category. Expectorant.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *guaiphenesin IPRS* or with the reference spectrum of guaiphenesin.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. Melts at 79° to 83° (2.4.21).

Tests

Appearance of solution. A 2.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 5.0 to 7.0, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 50.0 ml of *acetonitrile.*

Reference solution (a). A 0.001 per cent w/v solution of *guaiphenesin IPRS* in *acetonitrile.*

Reference solution (b). Dissolve 10 mg of *guaiacol* in 50.0 ml of *acetonitrile.* Dilute 0.5 ml of the solution to 50.0 ml with *acetonitrile.*

Reference solution (c). Dissolve 50 mg of *guaiacol* in 50.0 ml of *acetonitrile.* Dilute 5.0 ml of the solution to 10.0 ml with the test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 1 volume of *glacial acetic acid* and 99 volumes of *water,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
32	50	50
33	80	20
40	80	20

Inject reference solution (c) . The test is not valid unless the resolution between the peaks due to guaiphenesin and guaiphenesin impurity A is not less than 3.0. The relative retention time with reference to guaiphensin for 2-(2-methoxyphenoxy)propane-1,3-diol(B-isomer) (guaiphenesin impurity B) is about 0.9, for 2-methoxyphenol (guaiacol) (guaiphenesin impurity A) is about 1.4, for 1,1'-oxybis[3-(2-methoxyphenoxy)propan-2-ol] (bisether) (guaiphenesin impurity C) is about 3.1, for 1,3-bis(2-methoxyphenoxy)propan-2-ol (guaiphenesin impurity D) is about 3.7.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution the area of secondary peak corresponding to guaiphenesin impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of secondary peak corresponding to guaiphenesin impurity B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of area of all the secondary peaks other than guaiphenesin impurity B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Chlorides and monochlorohydrins. To 10 ml of a 2.0 per cent w/v solution, add 2 ml of *2 M sodium hydroxide,* heat on a water-bath for 5 minutes, cool and add 3 ml of *2 M nitric acid.* The resulting solution complies with the limit test for chlorides (2.3.12) using 2.0 ml of *chloride standard solution (25 ppm Cl)* (250 ppm).

Heavy metals (2.3.13). 12 ml of a solution prepared by dissolving 2.0 g in 25 ml of a mixture of 9 volumes of *ethanol (95 per cent)* and 1 volume of *water* complies with the limit test for heavy metals, Method D (25 ppm), using 10 ml of *lead standrd solution (2 ppm pb)*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

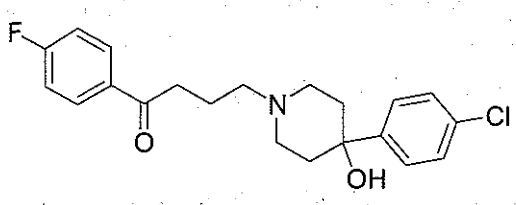
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 3 hours.

Assay. Weigh 50 mg and dissolve in 10 ml of *water*. Add 20 ml of *sodium periodate solution* and allow to stand for 10 minutes. Add 25.0 ml of *sodium arsenite solution* and 1 ml of a 16.6 per cent w/v solution of *potassium iodide*, allow to stand for 10 minutes and titrate with *0.05 M iodine solution* using 2 ml of *starch solution* as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of iodine required.

1 ml of *0.05 M iodine* is equivalent to 0.009911 g of $C_{10}H_{14}O_4$.

H

Haloperidol



$C_{21}H_{23}ClFNO_2$ Mol. Wt. 375.9

Haloperidol is 4-[4-(4-chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone.

Haloperidol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{23}ClFNO_2$, calculated on the dried basis.

Category. Antipsychotic.

Description. A white to faintly yellowish, amorphous or microcrystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *haloperidol IPRS* or with the reference spectrum of haloperidol.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in a mixture of 90 volumes of *methanol* and 10 volumes of *0.1 M hydrochloric acid* shows an absorption maximum at about 245 nm, about 0.49 to 0.53.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to haloperidol in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use and protect from light.

Test solution. Dissolve 0.1 g of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol.*

Reference solution (a). A solution containing 0.01 per cent w/v of *haloperidol IPRS* and 0.005 per cent w/v of *bromperidol IPRS* in *methanol.*

Reference solution (b). Dilute 5.0 ml of the test solution to 100.0 ml with *methanol.* Dilute 1.0 ml of the solution to 10.0 ml with *methanol.*

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a 1.7 per cent w/v solution of *tetrabutylammonium hydrogen sulphate*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
15	50	50
20	50	50
25	90	10

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to haloperidol and bromperidol is not less than 3.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

Assay. Dissolve 0.2 g in 25 ml of *anhydrous glacial acetic acid.* Titrate with *0.05 M perchloric acid*, using 0.2 ml of *1-naphtholbenzein solution* as indicator and titrating until the colour changes from orange-yellow to yellowish green. Carry out a blank titration.

1 ml of *0.05 M perchloric acid* is equivalent to 0.01879 g $C_{21}H_{23}ClFNO_2$.

Storage. Store protected from light.

Haloperidol Injection

Haloperidol Injection is a sterile solution of Haloperidol in Lactic Acid diluted with Water for Injections.

Haloperidol Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of haloperidol, $C_{21}H_{23}ClFNO_2$.

Usual strengths. 5 mg per ml; 10 mg per ml.

Identification

A. To a volume of the injection containing 20 mg of Haloperidol add 5 ml of *water* and 1 ml of *1 M sodium hydroxide* and extract with 10 ml of *chloroform*. Filter the chloroform extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *haloperidol IPRS* or with the reference spectrum of haloperidol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 245 nm.

Tests

pH (2.4.24). 2.8 to 3.6.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *chloroform*, 10 volumes of *glacial acetic acid* and 10 volumes of *methanol*.

Test solution. The injection under examination.

Reference solution (a). Dilute 1.0 ml of the injection to 100.0 ml with *methanol*.

Reference solution (b). Dilute 1.0 ml of the injection to 200.0 ml with *methanol*.

Apply to the plate a volume of the injection containing 0.1 mg of Haloperidol and the same volume of the reference solutions. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Bacterial endotoxins (2.2.3). Not more than 71.4 Endotoxin Units per mg of haloperidol.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume of the injection containing about 10 mg of Haloperidol add 8 ml of *water* and 10 ml of *1 M hydrochloric acid*. Extract with successive quantities of 25, 25, 10 and 10 ml of *ether*. Wash the combined ether extracts with 10 ml of *water*, combine the aqueous layers and remove the ether using a rotary evaporator. Add sufficient *water* to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with *methanol*. Measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of $C_{21}H_{23}ClFNO_2$ taking 346 as the specific absorbance at 245 nm.

Storage. Store protected from light.

Haloperidol Oral Solution

Haloperidol Oral Drops; Haloperidol Solution

Haloperidol Oral Solution is a solution of Haloperidol in Purified Water prepared with the aid of Lactic Acid.

Haloperidol Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of haloperidol, $C_{21}H_{23}ClFNO_2$.

Usual strengths. 1 mg per ml; 2 mg per ml.

Description. A clear, colourless solution.

Identification

A. To a volume of the oral solution containing 20 mg of Haloperidol, add 1 ml of *1 M sodium hydroxide*, extract with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *haloperidol IPRS* treated in the same manner or with the reference spectrum of haloperidol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 245 nm.

Tests

pH (2.4.24). 3.5 to 4.5.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 92 volumes of *dichloromethane*, 8 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution. Dilute the oral solution if necessary with *methanol* to contain 0.1 per cent w/v of Haloperidol.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with *methanol*.

Apply to the plate 50 µl of each solution. After development, dry the plate in air and spray with *dilute potassium*

iodobismuthate solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. To a measured volume of the oral solution containing about 10 mg of Haloperidol add 8 ml of *water* and 10 ml of *1 M hydrochloric acid*. Extract with successive quantities of 25, 25, 10 and 10 ml of *ether*. Wash the combined ether extracts with 10 ml of *water*, combine the aqueous layers and remove the ether using a rotary evaporator. Add sufficient *water* to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with *methanol*. Measure the absorbance of the resulting solution at the maximum at about 245 nm (2.4.7). Calculate the content of $C_{21}H_{23}ClFNO_2$ taking 346 as the specific absorbance at 245 nm.

Storage. Store protected from light at a temperature between 15° and 25°.

Haloperidol Tablets

Haloperidol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of haloperidol, $C_{21}H_{23}ClFNO_2$.

Usual strengths. 1.5 mg; 5 mg; 10 mg.

Identification

A. To a quantity of the powdered tablets containing 10 mg of Haloperidol add 5 ml of *water* and 1 ml of *1 M sodium hydroxide* and extract with 10 ml of *chloroform*. Filter the chloroform extract through absorbent cotton, evaporate the filtrate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *haloperidol IPRS* or with the reference spectrum of haloperidol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *gastric fluid simulated* (without enzyme),
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a suitable quantity of *haloperidol IPRS* in the dissolution medium to obtain a solution having known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 25 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *0.05M monobasic potassium phosphate*, adjusted to pH 4.0 with *1M sodium hydroxide* or *orthophosphoric acid* and 60 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{23}ClFNO_2$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{21}H_{23}ClFNO_2$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *chloroform*, 10 volumes of *glacial acetic acid* and 10 volumes of *methanol*.

Test solution (a). Shake a quantity of the powdered tablets containing 10 mg of Haloperidol with 10 ml of *chloroform*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *chloroform*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *chloroform*.

Reference solution (a). Dilute 1.0 ml of test solution (a) to 200.0 ml with *chloroform*.

Reference solution (b). A 0.1 per cent w/v solution of *haloperidol IPRS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (a) shows a distinct and clearly visible spot.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Place one tablet in 10 ml of the mobile phase, shake in an ultrasonic bath for 2 minutes, centrifuge and use the supernatant liquid after diluting suitably with the mobile phase if necessary.

Reference solution. A solution containing 0.015 per cent w/v of *haloperidol IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of a 1 per cent w/v solution of *ammonium acetate* and 45 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 247 nm,
- injection volume: 20 µl.

Calculate the content of $C_{21}H_{23}ClFNO_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the powder containing 20 mg of Haloperidol, shake with 60 ml of the mobile phase, place in an ultrasonic bath for 2 minutes, add sufficient quantity of the mobile phase to produce 100.0 ml. Centrifuge and use the supernatant liquid.

Reference solution. A solution containing 0.02 per cent w/v of *haloperidol IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of a 1 per cent w/v solution of *ammonium acetate* and 45 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 247 nm,
- injection volume: 20 µl.

Calculate the content of $C_{21}H_{23}ClFNO_2$ in the tablets.

Storage. Store protected from light.

Heparin Sodium

Heparin Sodium is the sodium salt of sulphated glycosaminoglycans present as a mixture of heterogeneous molecules varying in molecular weights. It is present in mammalian tissues and is usually obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by man. The sourcing of heparin material must be specified in compliance with applicable regulatory requirements. It is purified to retain a combination of activities against different fractions of the blood clotting sequence. It is composed of polymers of alternating derivatives of D-glucosamine (N-sulphated, O-sulphated, or N-acetylated) and uronic acid, L-iduronic acid or D-glucuronic acid) joined by glycosidic linkages. The component activities of the mixture are in ratios corresponding to those shown by the Heparin Sodium reference standard. Some of these components have the property of prolonging the clotting time of blood. This occurs mainly through the formation of a complex of each component with the plasma proteins antithrombin III and heparin cofactor II to potentiate the inactivation of thrombin. Other coagulation proteases in the clotting sequence, such as activated factor X, are also inhibited.

Heparin Sodium intended for use in the manufacture of parenteral preparation contains not less than 180 IU per mg for Heparin obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by man except bovine source. Heparin sodium intended for use in the manufacture of parenteral preparation contains not less than 150 IU per mg obtained from the intestinal mucosa or other suitable tissues of bovine and heparin sodium not intended for the use in the parenteral preparation contains not less than 120 IU per mg, calculated on the dried basis.

Category. Anticoagulant.

Description. A white or greyish-white powder; moderately hygroscopic.

Identification

A. It complies with the requirements described under assay.

B. It gives reaction (A) of sodium salts (2.3.1).

C. In the test for Oversulphated chondroitin sulphate, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 5.0 to 7.5, determined in 1.0 per cent w/v solution.

Oversulphated Chondroitin Sulphate (OSCS). Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 500 mg of the substance under examination in 10.0 ml of *water*.

Reference solution (a). A 2.0 per cent w/v solution of *heparin sodium IPRS* in *water*.

Reference solution (b). A 0.02 per cent w/v solution of *oversulphated chondroitin sulphate IPRS* in reference solution (a).

Chromatographic system
- a stainless steel column 25 cm x 2 mm, packed with a hydroxide- selective, strong anion-exchange resin consisting of a highly cross-linked core of 13 µm micoporous particles having a pore size less than 10 Angstrom units and consisting of ethylvinyl benzene cross-linked with 55 per cent divinylbenzene with a latex coating composed of 85 nm diameter microbeads bonded with alkanol quaternary ammonium ions (6 per cent),
- column temperature: 40°,
- mobile phase: A. a 0.04 per cent w/v solution of *sodium dihydrogen phosphate dihydrate* in *water*, adjusted to pH 3.0 with *phosphoric acid*, filter,
  B. a 14.0 per cent w/v solution of *sodium perchlorate* in mobile phase A, adjusted to pH 3.0 with *phosphoric acid*, filter,
- a gradient programme using the conditions given below,
- flow rate: 0.22 ml per minute,
- spectrophotometer set at 202 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
60	10	90
61	80	20
75	80	20

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to oversulphated chondroitin sulphate and heparin is not less than 1.5. The retention time of heparin is about 30 minutes and of oversulphated chondroitin sulphate is about 50 minutes.

Inject reference solution (a) and the test solution. The retention time of the principal peak obtained from the test solution corresponds to the peak obtained from reference solution (a). In the chromatogram obtained with the test solution, no peak corresponding to OSCS is observed.

Protein and nucleotidic impurities. Absorbance of a 0.4 per cent w/v solution at about 260 nm (2.4.7) (for nucleotides) and about 280 nm (for proteins) is not more than 0.2 and 0.15 respectively.

Nitrogen (2.3.30). Not more than 2.5 per cent, calculated on the dried basis.

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

Sulphated ash (2.3.18). 28.0 to 41.0 per cent, determined on 0.2 g.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying in an oven over phosphorous pentaoxide at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. *Potency.* For Heparin sodium obtained from the porcine or other source, perform the assay using method A. For Heparin Sodium obtained from bovine source, perform the assay using method A or B.

Method A

The anticoagulant activity of heparin is determined *in vitro* by its ability to accelerate the inhibition of thrombin, factor IIa (anti-IIa assay) by antithrombin. The International Unit is the activity contained in a stated amount of the International Standard for unfractionated heparin. *heparin sodium IPRS*, calibrated in International Units using by comparison with the International Standard using the 2 assays given below, is used as the reference preparation.

The assay of anti-factor Xa activity is carried out to determine the ratio of the anti-factor Xa activity to anti-factor IIa activity.

For anti-IIa and anti-Xa assays, carry out the assay by determining the absorbance (end-point method) or the change of absorbance per minute (kinetic method).

Anti-Factor IIa Activity

Reference and test solution. Prepare 4 independent series of 4 dilutions each of the substance under examination and of *heparin sodium IPRS* in *tris (hydroxylmethyl) aminomethane-EDTA buffer solution pH 8.4*; a concentration range within 0.005 IU and 0.03 IU per ml is suitable. The dilutions chosen must give a liner response when results are plotted as absorbance against log concentration.

Label 16 tubes for the dilutions of the substance under examination and 16 tubes for the dilutions of the reference preparation: T_1, T_2, T_3, T_4 for each of the 4 series of dilutions of the substance to be examined and S_1, S_2, S_3, S_4 for each of the 4 series of dilutions of the reference preparation. To each of the 32 tubes add 100 µl of *antithrombin III solution* and 50 µl of the appropriate dilution of the substance under examination or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in 2 subsequent series in the order S_1, S_2, S_3, S_4, T_1, T_2, T_3, T_4, T_1, T_2, T_3, T_4, S_1, S_2, S_3, S_4, allow to equilibrate at 37° (water-bath or heating block) for at least 1 minute and add to each tube 25 µl of *human thrombin solution*. Incubate for exactly 1 minute and add 50 µl of a chromogenic substrate specific to factor IIa at a concentration suitable for the assay (for example, *D-phenylalanyl-L-pipecolyl-L-arginine-4-nitroanilide dihydrochloride* dissolved in *water* to give a 1.25 mM solution).

For the kinetic method, transfer the mixtures to semi-micro cuvettes and measure the change in absorbance per minute (2.4.7) at 405 nm using a suitable reading device.

For the end-point method, stop the reaction after exactly 4 minutes by adding 50 µl of a 20 per cent v/v solution

of *glacial acetic acid*. Assess whether exactly 4 minutes of incubation with the chromogenic substrate yields the optimal absorbance reading and, if necessary, adjust the incubation time to give the best dose-response curve. Then, transfer the mixtures to semi-micro cuvettes and measure the absorbance (2.4.7) at 405 nm using a suitable reading device.

Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4* instead of the reference and test solutions; the 2 blank values do not differ significantly.

Calculate the regression of the absorbance on log concentrations of the solutions of the substance under examination and of *heparin sodium IPRS* and calculate the potency of the substance under examination in International Units per ml using the usual statistical methods for parallel-line assays (5.7).

Anti-Factor Xa Activity

Reference and test solution. Prepare 4 independent series of 4 dilutions each of the substance under examination and of *heparin sodium IPRS* in *tris(hydroxymethyl) aminomethane-EDTA buffer solution pH 8.4*; a concentration range must be within 0.03 IU and 0.375 IU per ml is suitable. The dilutions chosen must give a linear response when results are plotted as absorbance against log concentration.

Label 16 tubes for the dilutions of the substance under examination and 16 tubes for the dilutions of the reference preparation: T_1, T_2, T_3, T_4 for each of the 4 series of dilutions of the substance under examination and S_1, S_2, S_3, S_4 for each of the 4 series of dilutions of the reference preparation. To each of the 32 tubes add 50 µl of *antithrombin III solution* and 50 µl of the appropriate dilution of the substance under examination or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in 2 subsequent series in the order S_1, S_2, S_3, S_4, T_1, T_2, T_3, T_4, T_1, T_2, T_3, T_4, S_1, S_2, S_3, S_4, allow to equilibrate at 37° (water-bath or heating block) for 1 minute and add to each tube 100 µl of *bovine factor Xa solution*. Incubate for exactly 2 minutes and add 100 µl of a chromogenic substrate specific to factor Xa at a concentration suitable for the assay (for example, *N-α-benzyloxycarbonyl-D-arginyl-L-glycyl-L-arginine-4-nitroanilide-di-hydrochloride* dissolved in *water* to give a 1 mM solution).

For the kinetic method, transfer the mixtures to semi-micro cuvettes and measure the change in absorbance per minute (2.4.7) at 405 nm using a suitable reading device.

For the end-point method, stop the reaction after exactly 4 minutes by adding 50 µl of a 20 per cent v/v solution of *glacial acetic acid*. Assess whether exactly 4 minute of incubation with the chromogenic substrate yields the optimal absorbance reading and, if necessary, adjust the incubation time to give the best dose-response curve. Then, transfer the mixtures to semi-micro cuvettes and measure the absorbance (2.4.7) at 405 nm using a suitable reading device.

Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4* instead of the reference and test solutions; the 2 blank values do not differ significantly.

Calculate the regression of the absorbance on log concentrations of the solutions of the substance under examination and of *heparin sodium IPRS* and calculate the potency of the substance under examination in International Units per ml using the usual statistical methods for parallel-line assays (5.7).

Method B

Determine the potency of heparin sodium by comparing the concentration necessary to prevent the clotting of sheep or goat or human plasma with the concentration of the reference solution of heparin sodium necessary to give the same effect under the condition of the following method of assay.

Test solution. Dissolve 25 mg of substance under examination, in sufficient saline to produce a concentration of 1 mg per ml, and dilute to a concentration estimated to correspond to that of the reference solution.

Reference solution. Determine by preliminary trial, if necessary, approximately the minimum quantity of heparin sodium RS which, when added in 0.8 ml of saline, maintains fluidity in 1ml of prepared plasma for 1 hour after the addition of 0.2 ml of calcium chloride (1 in 100). This quantity is usually between 1 and 3 Heparin Units. On the day of the assay prepare a reference solution such that it contains, in each 0.8 ml of saline, the above-determined quantity of the reference standard.

Preparation of plasma. Collect blood from sheep directly into a vessel containing about 8 per cent of sodium citrate in the proportion of one volume to each 19 volumes of blood to be collected. Mix immediately by gentle agitation and inversion of the vessel. Promptly Centrifuge the blood, and pool the separated plasma. To a 1 ml portion of the pooled plasma in a clean test tube add 0.2 ml of calcium chloride (1 in 100), and mix. Consider the plasma suitable for use if a solid clot forms within 5 minutes. To store plasma for future use, subdivide the pooled lot into portions not exceeding 100 ml in volume, and store in the frozen state, preventing even partial thawing prior to use. For use in the assay, thaw the frozen plasma in a water-bath at a temperature not exceeding 37°. Remove particulate matter by straining the thawed plasma through a coarse filter.

Procedure. To meticulously clean 13 mm X 100 mm test tubes add graded amounts of the reference solution selecting the

amount so that the largest does not exceed 0.8 ml and so that they correspond roughly to a geometric series in which each step is approximately 5 per cent greater than the next lower. To each tube so prepared add sufficient saline to make the total volume 0.8 ml. Add 1.0 ml of prepared plasma to each tube. Then add 0.2 ml of calcium chloride (1 in 100), note the time, immediately insert a suitable stopper in each tube, and mix the contents by inverting three times in such a way that the entire inner surface of the tube is wet.

In the same manner set up a series using the test solution, completing the entire process of preparing and mixing the tubes of both reference solution and the test solution within 20 minutes after the addition of the prepared plasma. One hour, accurately timed, after the addition of the calcium chloride, determine the extent of clotting in each tube, recognizing three grades (0.25, 0.50, and 0.75) between zero and full clotting (1.0). If the series does not contain 2 tubes graded more than 0.5, and 2 tubes graded less than 0.5, repeat the assay, using appropriately modified reference solution and test solution.

Convert to logarithms the volumes of reference solution used in the successive 5 or 6 tubes that bracket a grade of clotting of 0.5, including at least 2 tubes with larger and 2 tubes with a smaller grade than 0.5. Number and list the tubes serially, and tabulate for each the grade of clotting observed in each tube. From the log-volumes, x, and separately from their corresponding grades of clotting, y, compute the paired averages x_i and y_i of Tubes 1, 2, and 3, of tubes 2, 3, and 4, of Tubes 3, 4, and 5, and, where the series consists of 6 tubes, of Tubes 4, 5, and 6, respectively. If for one of these paired averages the average grade, y_i, is exactly 0.50, the corresponding xi is the median log –volume of the reference solution x_s. Otherwise, interpolate xs from the paired values of y_i, x_i, and y_{i+1}, x_{i+1} that fall immediately below and above grade 0.5 as

$$x_s = \frac{x_i + (y_i - 0.5)(x_{i+1} - x_i)}{(y_i - y_{i+1})}$$

From the paired data on the tubes of the test solution, compute similarly its median log-volume x_U.

The log potency of the test solution is

$$M = x_s - x_u + \log R$$

where, R = v_s/v_u is the ratio of the heparin Units (v_s) per ml of the reference solution to the mg (v_U) of heparin sodium per ml of the test solution.

Heparin Sodium intended for use in the manufacture of parenteral preparation without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.03 Endotoxin Unit per Unit of heparin.

Heparin Sodium intended for use in the manufacture of parenteral preparation without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture in tightly-closed containers, sealed so as to exclude micro-organisms.

Labelling. The label states to indicate the tissue and the animal species from which it is derived, and the number of International Units per miligram.

Heparin Injection

Heparin Sodium Injection

Heparin Injection is a sterile solution of Heparin Sodium in Water for Injection. The pH of the solution may be adjusted by the addition of a suitable alkali or acid.

Heparin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated potency in terms of IU per ml.

Usual strengths. 1000 Units per ml; 5000 Units per ml; 10000 Units per ml.

Description. A clear, colourless or straw coloured solution, free from turbidity and matter which deposit on standing.

Identification

A. It complies with the requirements described under assay.

B. It gives reaction (A) of sodium salts (2.3.1).

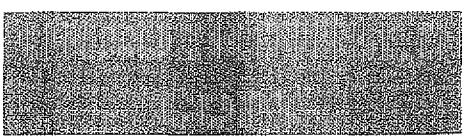
Tests

pH (2.4.24). 5.0 to 7.5.

Bacterial endotoxins (2.2.3). Not more than 0.03 Endotoxin Unit per Unit of heparin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *Potency.* The anticoagulant activity of heparin is determined *in vitro* by its ability to accelerate the inhibition of thrombin, factor IIa (anti-IIa assay) by antithrombin. The International Unit is the activity contained in a stated amount of the International Standard for unfractionated heparin. *heparin sodium IPRS*, calibrated in International Units using by comparison with the International Standard using the 2 assays given below, is used as the reference preparation.



The assay of anti-factor Xa activity is carried out to determine the ratio of the anti-factor Xa activity to anti-factor IIa activity.

For anti-IIa and anti-Xa assays, carry out the assay by determining the absorbance (end-point method) or the change of absorbance per minute (kinetic method).

Anti-Factor IIa Activity

Reference and test solution. Prepare 4 independent series of 4 dilutions each of the substance under examination and of *heparin sodium IPRS* in *tris (hydroxylmethyl) aminomethane-EDTA buffer solution pH 8.4*; a concentration range within 0.005 IU and 0.03 IU per ml is suitable. The dilutions chosen must give a liner response when results are plotted as absorbance against log concentration.

Label 16 tubes for the dilutions of the substance under examination and 16 tubes for the dilutions of the reference preparation: T_1, T_2, T_3, T_4 for each of the 4 series of dilutions of the substance to be examined and S_1, S_2, S_3, S_4 for each of the 4 series of dilutions of the reference preparation. To each of the 32 tubes add 100 µl of *antithrombin III solution* and 50 µl of the appropriate dilution of the substance under examination or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in 2 subsequent series in the order S_1, S_2, S_3, S_4, T_1, T_2, T_3, T_4, T_1, T_2, T_3, T_4, S_1, S_2, S_3, S_4, allow to equilibrate at 37° (water-bath or heating block) for at least 1 minute and add to each tube 25 µl of *human thrombin solution*. Incubate for exactly 1 minute and add 50 µl of a chromogenic substrate specific to factor IIa at a concentration suitable for the assay (for example, *D-phenylalanyl-L-pipecolyl-L-arginine-4-nitroanilide dihydrochloride* dissolved in *water* to give a 1.25 mM solution).

For the kinetic method, transfer the mixtures to semi-micro cuvettes and measure the change in absorbance per minute (2.4.7) at 405 nm using a suitable reading device.

For the end-point method, stop the reaction after exactly 4 minutes by adding 50 µl of a 20 per cent v/v solution of *glacial acetic acid*. Assess whether exactly 4 minutes of incubation with the chromogenic substrate yields the optimal absorbance reading and, if necessary, adjust the incubation time to give the best dose-response curve. Then, transfer the mixtures to semi-micro cuvettes and measure the absorbance (2.4.7) at 405 nm using a suitable reading device.

Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4* instead of the reference and test solutions; the 2 blank values do not differ significantly.

Calculate the regression of the absorbance on log concentrations of the solutions of the substance under examination and of *heparin sodium IPRS* and calculate the potency of the substance under examination in International Units per ml using the usual statistical methods for parallel-line assays (5.7).

Anti-Factor Xa Activity

Reference and test solution. Prepare 4 independent series of 4 dilutions each of the substance under examination and of *heparin sodium IPRS* in *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4*; a concentration range must be within 0.03 IU and 0.375 IU per ml is suitable. The dilutions chosen must give a linear response when results are plotted as absorbance against log concentration.

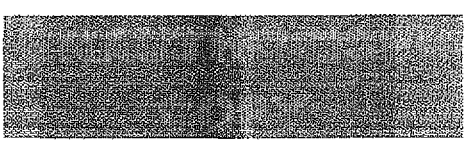
Label 16 tubes for the dilutions of the substance under examination and 16 tubes for the dilutions of the reference preparation: T_1, T_2, T_3, T_4 for each of the 4 series of dilutions of the substance under examination and S_1, S_2, S_3, S_4 for each of the 4 series of dilutions of the reference preparation. To each of the 32 tubes add 50 µl of *antithrombin III solution* and 50 µl of the appropriate dilution of the substance under examination or the reference preparation. After each addition, mix but do not allow bubbles to form. Treating the tubes in 2 subsequent series in the order S_1, S_2, S_3, S_4, T_1, T_2, T_3, T_4, T_1, T_2, T_3, T_4, S_1, S_2, S_3, S_4, allow to equilibrate at 37° (water-bath or heating block) for 1 minute and add to each tube 100 µl of *bovine factor Xa solution*. Incubate for exactly 2 minutes and add 100 µl of a chromogenic substrate specific to factor Xa at a concentration suitable for the assay (for example, *N-α-benzyloxycarbonyl-D-arginyl-L-glycyl-L-arginine-4-nitroanilide-di-hydrochloride* dissolved in *water* to give a 1 mM solution).

For the kinetic method, transfer the mixtures to semi-micro cuvettes and measure the change in absorbance per minute (2.4.7) at 405 nm using a suitable reading device.

For the end-point method, stop the reaction after exactly 4 minutes by adding 50 µl of a 20 per cent v/v solution of *glacial acetic acid*. Assess whether exactly 4 minute of incubation with the chromogenic substrate yields the optimal absorbance reading and, if necessary, adjust the incubation time to give the best dose-response curve. Then, transfer the mixtures to semi-micro cuvettes and measure the absorbance (2.4.7) at 405 nm using a suitable reading device.

Determine the blank amidolytic activity at the beginning and at the end of the procedure in a similar manner, using *tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4* instead of the reference and test solutions; the 2 blank values do not differ significantly.

Calculate the regression of the absorbance on log concentrations of the solutions of the substance under examination and of *heparin sodium IPRS* and calculate the potency of the substance under examination in International Units per ml using the usual statistical methods for parallel-line assays (5.7).

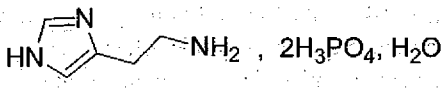


Storage. Store at a temperature not exceeding 30°.

Labelling. The label states to indicate the volume of the total contents and the potency in terms of Heparin Units per ml, except that single-dose containers may be labeled additionally to indicate the single unit-dose volume and the total number of Heparin Units. Where it is labeled with total content, the label states also that the entire contents are to be used or, if not, any remaining portion is to be discarded. Label it to indicate also the tissue and the animal species from which it is derived.

Histamine Phosphate

Histamine Acid Phosphate



$C_5H_9N_3,2H_3PO_4,H_2O$ Mol. Wt. 325.2

Histamine Phosphate is 2-(1*H*-imidazol-4-yl)ethylamine diphosphate monohydrate.

Histamine Phosphate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_5H_9N_3,2H_3PO_4$, calculated on the anhydrous basis.

Category. Diagnostic aid (gastric secretion indicator).

Description. Colourless, long prismatic crystals.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *histamine phosphate IPRS* or with the reference spectrum of histamine phosphate.

B. In the test for Histidine, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 7 ml of *water* and add 3 ml of *sodium hydroxide solution*. Dissolve 50 mg of *sulphanilic acid* in 10 ml of *water* containing 0.1 ml of *hydrochloric acid* and 0.1 ml of a 10 per cent w/v solution of *sodium nitrite*. On mixing the two solutions a deep red colour is produced.

D. It gives reaction (A) of phosphates (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

pH (2.4.24). 3.7 to 3.9, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A).

Histidine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 75 volumes of *acetonitrile*, 20 volumes of *water* and 5 volumes of *strong ammonia solution*.

Test solution (a). Dissolve 0.5 g of the substance under examination in 10 ml of *water*.

Test solution (b). Dilute 5.0 ml of test solution to 25.0 ml with *water*.

Reference solution (a). A 1.0 per cent w/v solution of *histamine phosphate IPRS*.

Reference solution (b). A solution containing 0.05 per cent w/v of *DL-histidine monohydrochloride*.

Reference solution (c). A mixture of equal volumes of test solution (a) and reference solution (b).

Apply to the plate 1 µl of each solution. After development, dry the plate in a current of air and repeat the development in the same direction. Dry the plate in a current of air, spray with *ninhydrin solution* and heat at 110° for 10 minutes. Any spot corresponding to histidine monohydrochloride in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Sulphates (2.3.17). 3 ml of solution A diluted to 15 ml with *water* complies with the limit test for sulphates (0.1 per cent).

Water (2.3.43). 5.0 to 6.2 per cent, determined on 0.3 g.

Assay. Weigh 0.14 g, dissolve in 5 ml of *anhydrous formic acid* and add 20 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01536 g of $C_5H_9N_3,2H_3PO_4$.

Storage. Store protected from light.

Histamine Phosphate Injection

Histamine Acid Phosphate Injection

Histamine Phosphate Injection is a sterile solution of Histamine Phosphate in Water for Injections.

Histamine Phosphate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of histamine phosphate, $C_5H_9N_3,2H_3PO_4,H_2O$.

Usual strength. 1 mg per ml.

Identification

A. Evaporate a volume of the injection containing about 2 mg of Histamine Phosphate on a water bath to dryness, dissolve

the residue in 0.5 ml of *water*, and add 0.5 ml of *sodium hydroxide*. Add 2 drops of a 10 per cent w/v solution of *sodium nitrite* and 1 ml of a solution prepared by mixing 50 mg of *sulphanilic acid* with 10 ml of *water* containing 2 drops of *hydrochloric acid*; an orange-red colour is produced.

B. To 1 ml of the injection containing not less than 1 mg of Histamine Phosphate (concentrate a larger volume by evaporation, if necessary), add *ammonium molybdate* solution dropwise; a yellow precipitate, which is soluble in *ammonia*, is formed.

Tests

pH (2.4.24). 3.0 to 6.0.

Bacterial endotoxins (2.2.3). Not more than 125.0 Endotoxin Units per mg of histamine phosphate.

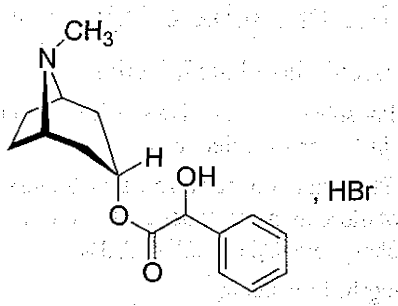
Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Measure a volume of the injection containing about 10 mg of Histamine Phosphate, transfer to a tared 25 ml centrifuge tube containing a thin glass rod slightly curved at the end, add 0.5 ml of *nitranilic acid solution* with continuous stirring and allow to stand for 15 minutes. Add 10 ml of *ethanol (95 per cent)*, mix and keep at 0° for 3 hours. Centrifuge for 1 minute, dislodge any particles at the surface and again centrifuge for 1 minute. Decant the supernatant liquid and stir the precipitate with 5 ml of ice-cold *ethanol (95 per cent)*. Centrifuge for 2 minutes, decant and repeat the washing with two further quantities, each of 5 ml, of ice-cold *ethanol (95 per cent)* and finally with 5 ml of *ether*. Smear the residue over the inside of the tube by means of the glass rod and dry to constant weight at 130°.

1 g of the residue is equivalent to 0.9529 g of $C_5H_9N_3,2H_3PO_4,H_2O$.

Storage. Store protected from light.

Homatropine Hydrobromide



$C_{16}H_{21}NO_3,HBr$ Mol. Wt. 356.3

Homatropine Hydrobromide is (1*R*,3*r*,5*S*)-3-(*RS*)-mandeloyloxytropane hydrobromide.

Homatropine Hydrobromide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{21}NO_3,HBr$, calculated on the dried basis.

Category. Anticholinergic.

Description. Colourless crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *homatropine hydrobromide IPRS* or with the reference spectrum of homatropine hydrobromide.

B. Dissolve 50 mg in 1 ml of *water* and add 2 ml of *2 M acetic acid*. Heat, add 4 ml of *picric acid solution* and allow to cool, shaking occasionally. The crystals, after washing with two quantities, each of 3 ml, of iced *water* and drying at 105° melt at 182° to 186° (2.4.21).

C. Dissolve about 10 mg in 1 ml of *water*, add a slight excess of *10 M ammonia* and shake with 5 ml of *chloroform*. Evaporate the chloroform layer to dryness on a water-bath and add 1.5 ml of a 2 per cent w/v solution of *mercuric chloride* in *ethanol (60 per cent)*; a yellow colour develops which becomes red on warming.

D. It gives reaction (A) of bromides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BS8 (2.4.1).

pH (2.4.24). 5.0 to 6.5; determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 25.0 ml with the mobile phase.

Reference solution (c). A 0.01 per cent w/v solution of *hyoscine hydrobromide IPRS* in the mobile phase. To 10.0 ml of the solution, add 0.5 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,

- mobile phase: a mixture of 33 volumes of *methanol* and 67 volumes of a solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* and 7.0 g of *sodium heptanesulphonate monohydrate* in 1000 ml of *water*, adjusted to pH 2.7 with 33 per cent w/v solution of *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Name	Relative retention time
Homatropine impurity C[1]	0.2
Homatropine impurity A[2]	0.9
Homatropine (Retention time: about 6.8 minutes)	1.0
Homatropine impurity B[3]	1.1
Homatropine impurity D[4]	1.9

[1]mandelic acid,
[2]dehydrohomatropine,
[3]hyoscine,
[4]atropine.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to homatropine and homatropine impurity B is not less than 1.5 and the tailing factor for principal peak is not more than 2.5.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to homatropine impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of any peak corresponding to homatropine impurities B, C and D is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) and the peak due to the bromide ion which appears close to the peak due to the solvent.

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 0.5 g.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.3 g in 20 ml of *anhydrous glacial acetic acid* and add 7 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03563 g of $C_{16}H_{21}NO_3,HBr$.

Storage. Store protected from light.

Homatropine Eye Drops

Homatropine Hydrobromide Eye Drops

Homatropine Eye Drops are a sterile solution of Homatropine Hydrobromide in Purified Water.

Homatropine Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of homatropine hydrobromide, $C_{16}H_{21}NO_3,HBr$.

Usual strength. 2 per cent w/v.

Identification

A. To a volume containing 60 mg of Homatropine Hydrobromide add 3 ml of *dilute ammonia solution*, extract with 15 ml of *chloroform*, dry the chloroform extract over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *homatropine hydrobromide IPRS* treated in the same manner or with the reference spectrum of homatropine.

B. To the residue obtained in test A, add 1.5 ml of a 2 per cent w/v solution of *mercuric chloride* in *ethanol (60 per cent)*; a yellow colour is produced which becomes red on gentle warming (distinction from most other alkaloids except atropine and hyoscyamine).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 134 volumes of *ethyl acetate*, 33 volumes of *anhydrous formic acid* and 33 volumes of *water*.

Test solution. Use the eye drops, diluted if necessary with water to contain 1 per cent w/v of Homatropine Hydrobromide.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with *water*.

Apply to the plate 40 µl of each solution. After development, dry the plate at 105° until the odour of the solvent is no longer detectable, allow to cool and spray with *dilute potassium iodobismuthate solution* until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. After development, dry at 105° until the odour of solvent is no longer detectable, allow to cool and spray with *dilute potassium iodobismuthate solution*

until spots appear. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). Add 1 ml of a 2 per cent w/v solution of *atropine sulphate IPRS* (internal standard) in *methanol* (solution A) and 1 ml of *dilute ammonia solution* to a volume of the eye drops containing about 20 mg of Homatropine Hydrobromide, diluted if necessary to 5.0 ml with *water*. Extract with two quantities, each of 5 ml, of *chloroform*, shake the combined extracts with 1 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 10.0 ml of *dichloromethane*. To 1.0 ml of the solution add 0.2 ml of a mixture of 4 volumes of N,O-*bis* (*trimethylsilyl*)-*acetamide* and 1 volume of *trimethyl- chlorosilane*, mix and allow to stand for 30 minutes.

Test solution (b). Prepare in the same manner as test solution (a) but omitting the addition of solution A.

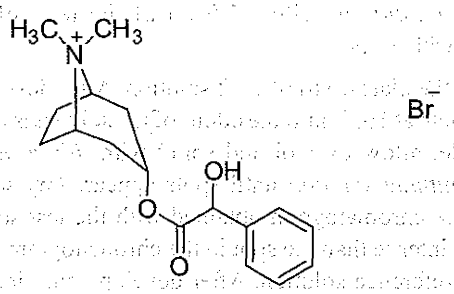
Reference solution. Add 1 ml of solution A and 1 ml of *dilute ammonia solution* to 5.0 ml of a 0.4 per cent w/v solution of *homatropine hydrobromide IPRS* and complete the procedure described under test solution (a) beginning at the words "Extract with two quantities, each of 5 ml, of *chloroform*....".

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (Such as OV-17),
- temperature:
  column. 220°,
  inlet port and detector. 280°,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Calculate the content of $C_{16}H_{21}NO_3$,HBr in the eye drops.

Homatropine Methylbromide



$C_{17}H_{24}BrNO_3$ Mol. Wt. 370.3

Homatropine Methylbromide is (1*R*,3*r*,5*S*)-3-[[(2*RS*)-2-hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide.

Homatropine Methylbromide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{17}H_{24}BrNO_3$, calculated on the dried basis.

Category. Anticholinergic

Description. A white or almost white, crystalline powder or colourless crystals.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *homatropine methylbromide IPRS* or with the reference spectrum of homatropine methylbromide.

B. Dissolve 50 mg in 1 ml of *water* and add 2 ml of *dilute acetic acid*. Heat and add 4 ml of *picric acid solution*, allow to cool, shaking occasionally. Wash the crystals, with two quantities, each of 3 ml of iced *water* and dried at 105°. The residue melts between 132° to 138° (2.4.21).

C. It gives reaction (A) of bromides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 4.5 to 6.5, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 18 volumes of *acetonitrile* and 82 volumes of mobile phase A.

Test solution. Dissolve 50 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution (a). Dilute 5.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 25.0 ml with the solvent mixture.

Reference solution (c). Dissolve 5.0 mg of *homatropine hydrobromide IPRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. To 10.0 ml of the solution, add 0.5 ml of the test solution and dilute to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),

- column temperature: 25°,
- mobile phase: A. dissolve 3.4 g of *potassium dihydrogen phosphate* and 5 g of *sodium heptanesulphonate monohydrate* in 1000 ml of *water*, and adjusted to pH 3.0 with a 33 per cent w/v solution of *orthophosphoric acid*,

  B. a mixture of 40 volumes of mobile phase A and 60 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
2	70	30
15	30	70
20	70	30
25	70	30

Inject reference solution (c). The test is not valid unless resolution between the peaks due to homatropine methylbromide and homatropine impurity B is not less than 2.5 and tailing factor for the peak due to homatropine methylbromide is not more than 2.5. The relative retention time with respect to homatropine methylbromide for (*2RS*)-2-hydroxy-2-phenylacetic acid (mandelic acid) (homatropine impurity C) is about 0.7, for (*1R,3s,5s*)-3-[[(*2RS*)-2-hydroxy-2-phenylacetyl]oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]oct-6-ene(methyldehydro-homatropine) (homatropine impurity A) is about 0.9, for homatropine (homatropine impurity B) is about 1.2, for (*1R,2R,4S,5S,7s*)-7-[[(*2S*)-3-hydroxy-2-phenyl propanoyl]oxy-9,9-dimethyl-3-oxa-9-azoniatricyclo nonane (methylhyoscine) (homatropine impurity D) is about 1.3, for methylatropine (homatropine impurity E) is about 1.4 and for methyl (*2RS*)-2-hydroxy-2-phenylacetate(methyl mandelate) (homatropine impurity F) is about 1.7.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution the area of secondary peak corresponding to homatropine impurities A and B; for each impurity is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), The area of the secondary peaks corresponding to homatropine impurities C, D, E and F; for each impurities is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore the peak due to the bromide ion which appears close to the principle peak due the solvent. Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.3 g and dissolve in 10 ml of *water*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25), using a silver indicator electrode and a silver- silver chloride reference electrode.

1 ml of *0.1 M silver nitrate* is equivalent to 0.03703 g of $C_{17}H_{24}BrNO_3$.

Storage. Store protected from light.

Homatropine Methylbromide Tablets

Homatropine Methylbromide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of homatropine methylbromide, $C_{17}H_{24}BrNO_3$.

Usual strength. 15 mg.

Identification

Shake a quantity of powdered tablets containing about 10 mg of Homatropine Methylbromide, with 15 ml of a mixture of equal volumes of *methanol* and *water* for 10 minutes, and filter. Evaporate the filtrate on a steam bath to dryness, and dry at 105° for 1 hour. The residue of homatropine methylbromide so obtained melts between 190° and 198° (2.4.21).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted if necessary with dissolution medium at 258 nm. Calculate the content of homatropine methylbromide, $C_{17}H_{24}BrNO_3$ in the medium from the absorbance obtained from a solution of known concentration of *homatropine methylbromide IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{17}H_{24}BrNO_3$.

Uniformity of content.

Test solution. Disperse 1 tablet in *water* to obtain a solution of 0.01 per cent w/v of Homatropine Methylbromide.

Reference solution. A 0.01 per cent w/v solution of *homatropine methylbromide IPRS* in *water.*

Transfer 2.0 ml each of the test solution and the reference solution to separate glass-stoppered 50-ml flasks. To each flask, add 0.1 ml of 10 per cent w/v solution of *sodium hydroxide solution* and heat on a water-bath at 80° for 15 minutes, cool. Add 2.0 ml of *0.2 M ceric ammonium sulphate* in *1 M sulphuric acid*, and mix. To each flask, add 20 ml of *n-hexane* and shake for 15 minutes and measure the absorbance at 242 nm (2.4.7).

Other tests. Comply with the tests stated under Tablets.

Assay

Test solution. Weigh and powder 20 tablets. Shake a quantity of powder containing about 12.5 mg of Homatropine Methylbromide with 10 ml of *water* for 30 minutes. Filter under reduced pressure through a sintered-glass crucible into a test tube placed in the suction flask under the filtering funnel, and wash under suction with several small portions of *water*. Transfer the contents of the test tube to a 25-ml volumetric flask and dilute with *water*.

Reference solution. A 0.05 per cent w/v solution of *homatropine methylbromide IPRS* in *water.*

Transfer 10.0 ml each of the test solution and the reference solution to separate test tubes, to each add 1 ml of *2.5 M sulphuric acid* and 2 ml of *ammonium reineckate*, shake and allow to stand for 1 hour. Filter through a sintered-glass crucible with suction, using portions of the filtrate to transfer the precipitate completely to the filter, and wash it with three 2-ml portions of ice-cold *water*. Completely dissolve the precipitate by pouring over it 1-ml portions of *acetone* with the application of suction and dilute to 10 ml with *acetone* and measure the absorbance at 525 nm (2.4.7).

Calculate the content of $C_{17}H_{24}BrNO_3$ in the tablets.

Storage. Store protected from light.

Hyaluronidase

Hyaluronidase is a material containing enzymes, which depolymerise the mucopolysaccharide, hyaluronic acid. It may be prepared from the testes and semen of mammals and purified by fractional precipitation so as to remove inert material and to which hydrolysed gelatin or a suitable non-protein stabilising agent may be added. The product is freeze-dried in single dose containers, which are sealed so as to exclude micro-organisms.

Hyaluronidase contains not less than 300 Units per mg, calculated on the dried basis. It may contain a suitable stabilizer.

Category. Depolymerising enzyme used as spreading factor.

Description. A white or yellowish-white, fluffy powder.

Identification

A. A solution containing the equivalent of 100 Units in 1 ml of *saline solution* depolymerises an equal volume of a 1 per cent w/v solution of *sodium hyaluronate* at 20° in 1 minute as shown by a pronounced decrease in viscosity. This action is destroyed by heating the initial solution at 100° for 30 minutes.

B. A solution containing the equivalent of 1 Unit in 0.2 ml of *saline solution* when injected intracutaneously into experimental animals together with a suitable indicator shows a spreading activity when compared with a control solution.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more than faintly yellow.

pH (2.4.24). 4.5 to 7.5, determined in a 0.3 per cent w/v solution in *carbon dioxide-free water.*

Light absorption. Dissolve a quantity containing 1500 Units in sufficient *carbon dioxide-free water* to produce 5.0 ml and measure the absorbance of the resulting solution at about 260 nm and 280 nm; absorbance at about 260 nm, not more than 0.42 and at about 280 nm, not more than 0.60 (2.4.7).

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by drying in vacuum at 60° for 2 hours.

Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per unit of hyaluronidase.

Sterility (2.2.11). Complies with the test for sterility.

Assay. The potency of hyaluronidase is determined by comparing its effects against those of the Standard Preparation.

Diluent for hyaluronidase solution. Mix 100 ml of *phosphate buffer solution pH 6.4* with 100 ml of *water*. Dissolve 0.14 g of hydrolysed gelatin in the solution at 37°, used within 2 hours.

Standard Preparation

The Standard Preparation is the 1st International Standard for Hyaluronidase, bovine, established in 1955, consisting of dried bovine testicular hyaluronidase diluted with lactose (supplied in ampoules containing 10 tablets of 20 mg each; each tablet contains approximately 200 Units).

Test solution. Dissolve a suitable quantity of the preparation under examination by adding cold *diluent for hyaluronidase solutions*. Dilute the solution with cold *diluent for hyaluronidase solutions* so that the absorbances of the

dilutions being assayed will fall on the upper linear part of the reference curve prepared as follows.

To each of 12 test-tubes (100 mm x 16 mm) add 0.50 ml of *hyaluronate solution* and, respectively and in duplicate, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 ml of *diluent for hyaluronidase solutions*. If quantities of the solution of the standard solution other than those indicated below are used, change the quantities of *diluent for hyaluronidase solutions* accordingly. At intervals of 30 seconds add to the tubes 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of the solution of the standard solution, respectively and in duplicate, making the final volume in each tube 1.0 ml, mixing the contents by shaking gently and placing each tube in a water-bath maintained at 37.0° ± 0.2°. After exactly 30 minutes, remove each tube in order from the water-bath at intervals of 30 seconds and immediately add 4.0 ml of *serum solution*. Shake and allow to stand at room temperature for 30 minutes. Shake again and measure the absorbance at about 640 nm (2.4.7). Repeat the operation using 0.50 ml of *phosphate-buffered saline* in place of the *hyaluronate solution* and make any necessary corrections. Prepare a reference curve by plotting the mean of the corrected absorbance for each level against the potency.

Standard solution. Dissolve one tablet of the Standard Preparation, weighed, in sufficient cold *diluent for hyaluronidase solutions* to give a solution of known concentration containing about 1.5 Units per ml. This solution should be prepared immediately before use.

To each of 6 test-tubes (100 mm x 16 mm) add 0.5 ml of *hyaluronate solution* and sufficient *diluent for hyaluronidase solutions* so that the final volume in each tube after the addition of the solution of the preparation being examined is 1.0 ml. At intervals of 30 seconds add to each tube sufficient of the solution of the preparation being examined so that the tubes contain about 0.3, 0.5 and 0.7 Units, respectively and in duplicate, shaking each tube gently and continuing as described under test solution, beginning at the words "placing each tube in a water-bath..."

Storage. Store protected from moisture at a temperature not exceeding 15°.

Labelling. The label states (1) the total number of Units in the container; (2) the name of any added stabilising agent; (3) that the preparation is not intended for intravenous injection.

Hyaluronidase Injection

Hyaluronidase Injection is a sterile material consisting of Hyaluronidase with or without excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Hyaluronidase Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated number of Units of hyaluronidase activity.

Usual strength. 1500 Units.

Description. A white or yellowish-white powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. A solution containing the equivalent of 100 Units in 1 ml of *saline solution* depolymerises an equal volume of a 1 per cent w/v solution of *sodium hyaluronate* at 20° in 1 minute as shown by a pronounced decrease in viscosity. This action is destroyed by heating the initial solution at 100° for 30 minutes.

B. A solution containing the equivalent of 1 Unit in 0.2 ml of *saline solution* when injected intracutaneously into experimental animals together with a suitable indicator shows a spreading activity when compared with a control solution.

Tests

pH (2.4.24). 4.5 to 7.5, determined in a 0.3 per cent w/v solution in *carbon dioxide-free water*.

Appearance of solution. A 1.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more than faintly yellow.

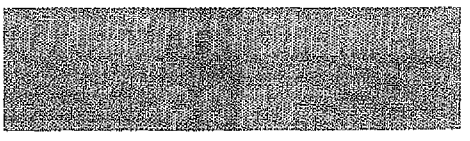
Bacterial endotoxins (2.2.3). Not more than 0.2 Endotoxin Unit per unit of hyaluronidase.

Assay. The potency of hyaluronidase is determined by comparing its effects against those of the Standard Preparation.

Diluent for hyaluronidase solution. Mix 100 ml of *phosphate buffer solution pH 6.4* with 100 ml of *water*. Dissolve 0.14 g of hydrolysed gelatin in the solution at 37°, used within 2 hours.

Standard Preparation

The Standard Preparation is the 1st International Standard for Hyaluronidase, bovine, established in 1955, consisting of dried



bovine testicular hyaluronidase diluted with lactose (supplied in ampoules containing 10 tablets of 20 mg each; each tablet contains approximately 200 Units).

Test solution. Dissolve the contents of a container by adding cold *diluent for hyaluronidase solutions*. Dilute the solution with cold *diluent for hyaluronidase solutions* so that the absorbances of the dilutions being assayed will fall on the upper linear part of the reference curve prepared as follows.

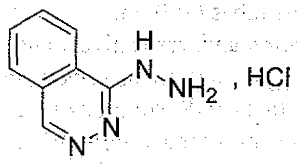
To each of 12 test-tubes (100 mm x 16 mm) add 0.50 ml of *hyaluronate solution* and, respectively and in duplicate, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.0 ml of *diluent for hyaluronidase solutions*. If quantities of the solution of the standard solution other than those indicated below are used, change the quantities of *diluent for hyaluronidase solutions* accordingly. At intervals of 30 seconds add to the tubes 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of the solution of the standard solution, respectively and in duplicate, making the final volume in each tube 1.0 ml, mixing the contents by shaking gently and placing each tube in a water-bath maintained at 37.0° ± 0.2°. After exactly 30 minutes, remove each tube in order from the water-bath at intervals of 30 seconds and immediately add 4.0 ml of *serum solution*. Shake and allow to stand at room temperature for 30 minutes. Shake again and measure the absorbance at about 640 nm (2.4.7). Repeat the operation using 0.50 ml of *phosphate-buffered saline* in place of the *hyaluronate solution* and make any necessary corrections. Prepare a reference curve by plotting the mean of the corrected absorbance for each level against the potency.

Standard solution. Dissolve one tablet of the Standard Preparation, accurately weighed, in sufficient cold *diluent for hyaluronidase solutions* to give a solution of known concentration containing about 1.5 Units per ml. This solution should be prepared immediately before use.

To each of 6 test-tubes (100 mm x 16 mm) add 0.50 ml of *hyaluronate solution* and sufficient *diluent for hyaluronidase solutions* so that the final volume in each tube after the addition of the solution of the preparation being examined is 1.0 ml. At intervals of 30 seconds add to each tube sufficient of the solution of the preparation being examined so that the tubes contain about 0.3, 0.5 and 0.7 Units, respectively and in duplicate, shaking each tube gently and continuing as described under test solution, beginning at the words "placing each tube in a water-bath..."

Labelling. The label states (1) the total number of Units contained in it; (2) the nature of any added stabilising agent; (3) that the injection should be used immediately after preparation; (4) that the preparation is not intended for intravenous injection.

Hydralazine Hydrochloride



$C_8H_8N_4,HCl$ Mol. Wt. 196.6

Hydralazine Hydrochloride is phthalazin-1-yl-hydrazine hydrochloride.

Hydralazine Hydrochloride contains not less 98.5 per cent and not more than 101.0 per cent of $C_8H_8N_4,HCl$, calculated on the dried basis.

Category. Vasodilator; antihypertensive.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydralazine hydrochloride IPRS* or with the reference spectrum of hydralazine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution shows absorption maxima at about 240 nm, 260 nm, 305 nm and 315 nm and their absorbances are about 0.55, 0.55, 0.27 and 0.22 respectively.

C. Dissolve 0.5 g in a mixture of 8 ml of *2 M hydrochloric acid* and 100 ml of *water*. Add 2.0 ml of *sodium nitrite solution*, allow to stand for 10 minutes and filter. The precipitate, after washing with *water* and drying at 105°, melts at 209° to 212° (2.4.21).

D. To a solution of about 10 mg in 2 ml of *water* add 2 ml of a 2 per cent w/v solution of *2-nitrobenzaldehyde* in *ethanol (95 per cent)*; an orange precipitate is obtained.

E. A 5.0 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. A 0.4 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution GYS4 (2.4.1).

pH (2.4.24). 3.5 to 4.2, determined in a 2.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 25 mg of the substance under examination in sufficient quantity of the mobile phase to make 50 ml.

Test solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 5.0 ml of test solution (b) to 25.0 ml with the mobile phase.

Reference solution (b). Dissolve 25 mg of *phthalazine* in sufficient quantity of the mobile phase to make 50 ml and dilute 4.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). Mix 4.0 ml of test solution (a) and 10.0 ml of reference solution (b) and dilute to 100.0 ml with the mobile phase.

The solutions should be used within 8 hours of preparation.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous spherical particles of finely-divided silica gel chemically bonded to nitrile groups (10 µm),
- mobile phase: a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 1.44 g of *sodium dodecyl sulphate* and 0.75 g of *tetrabutylammonium bromide* per litre adjusted to pH 3.0 with *0.05 M sulphuric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject test solution (b) and adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram is not less than 70 per cent of the full scale of the recorder. When the chromatograms are recorded in the prescribed conditions, the retention time of hydralazine is about 10 to 12 minutes. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject test solution (a) and continue the chromatography for 3 times the retention time of hydralazine. Inject reference solution (a). The area of any secondary peak in the chromatogram obtained with test solution (a) is not greater than the area of the peak in the chromatogram obtained with reference solution (a).

The test is not valid unless (a) the chromatogram obtained with reference solution (c) shows two principal peaks and the resolution between the peaks is not less than 2.5 and (b) the principal peak in the chromatogram obtained with reference solution (a) has a signal-to-noise ratio of at least 3.

Hydrazine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (95 per cent)*.

Test solution. Dissolve 0.12 g of the substance under examination in 4 ml of *water* and 4 ml of a 15 per cent w/v solution of *salicylaldehyde* in *methanol* and 0.2 ml of *hydrochloric acid*. Mix and let it stand at a temperature not exceeding 25° for 2 to 4 hours to allow complete sedimentation of the precipitate. Add 4 ml of toluene, shake vigorously and centrifuge. Transfer the supernatant liquid to a 100-ml separating funnel, separate the toluene layer and shake vigorously, each time for 3 minutes, with two quantities, each of 20 ml, of a 20 per cent w/v solution of *sodium metabisulphite* and with two quantities, each of 50 ml, of *water*. Separate the toluene layer and use it as the test solution.

Reference solution. Prepare at the same time and in the same manner as described for the test solution using 1 ml of a solution prepared by dissolving 12 mg of *hydrazine sulphate* in sufficient quantity of *2 M hydrochloric acid* to make 100 ml and diluting 1.0 ml of the solution to 100.0 ml with the same solvent and 3 ml of *water*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine under ultraviolet light at 365 nm. Any spot in the chromatogram obtained with the test solution showing a yellow fluorescence is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). Moisten the residue obtained in the test for Sulphated ash with 2 ml of *hydrochloric acid*, evaporate to dryness and dissolve the residue in sufficient *water* to produce 20 ml. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (2 ppm Pb)* to prepare the standard

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa.

Assay. Weigh 0.1 g and dissolve in a mixture of 25 ml of water and 35 ml of *hydrochloric acid*. Titrate with *0.05 M potassium iodate*, determining the end-point potentiometrically (2.4.25) and using a calomel reference electrode and a platinum indicator electrode.

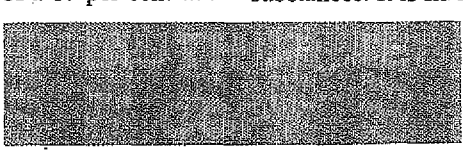
1 ml of *0.05 M potassium iodate* is equivalent to 0.009832 g of $C_8H_8N_4,HCl$.

Storage. Store protected from light.

Hydralazine Injection

Hydralazine Hydrochloride Injection

Hydralazine Injection is a sterile material consisting of Hydralazine Hydrochloride with or without auxiliary substances. It is filled in a sealed container.



The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use. For intravenous infusion, the injection should be diluted with an appropriate volume of a suitable diluent.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Hydralazine Injection contains not less than 98.0 per cent and not more than 114.0 per cent of the stated amount of hydralazine hydrochloride, $C_8H_8N_4,HCl$.

Usual strength. 20 mg.

Description. A white or almost white powder; very hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydralazine hydrochloride IPRS* or with the reference spectrum of hydralazine hydrochloride.

B. It give the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 4.2, determined in a 2.0 per cent w/v solution.

Appearance of solution. A 2.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1). A 2.0 per cent w/v solution in *0.01 M hydrochloric acid* is not more intensely coloured than reference solution GYS6 (2.4.1).

Hydrazine. Determine by thin-layer chromatography (2.4.17), using a silica gel 60-precoated plate.

Mobile phase. The upper layer obtained by shaking together 80 volumes of *hexane*, 20 volumes of *strong ammonia solution* and 20 volumes of *ethyl acetate*.

Test solution. Dissolve the contents of a container in sufficient *0.1 M methanolic hydrochloric acid* to produce a solution containing 0.5 per cent w/v of Hydralazine Hydrochloride. To 2.0 ml add 1.0 ml of a 2 per cent w/v solution of *salicylaldehyde* in *methanol* and 0.1 ml of *hydrochloric acid*, centrifuge and decant the supernatant liquid.

Reference solution. Prepare in the same manner, but using 2.0 ml of a 0.00025 per cent w/v solution of *hydrazine sulphate* in *0.1 M methanolic hydrochloric acid* in place of the solution of the substance under examination.

Apply to the plate 40 µl of each solution. After development, dry the plate in air and spray with *dimethylaminobenzaldehyde reagent*. In the chromatogram obtained with the test solution, any spot corresponding to hydrazine is not more intense than the spot in the chromatogram obtained with the reference solution.

Bacterial endotoxins (2.2.3). Not more than 1.45 Endotoxin Units per mg of hydralazine.

Assay. Mix the contents of 10 containers. Dissolve 0.1 g of the mixed contents of the 10 containers in a mixture of 25 ml of *water* and 35 ml of *hydrochloric acid*. Titrate with *0.05 M potassium iodate*, determining the end-point potentiometrically (2.4.25) and using a calomel reference electrode and a platinum indicator electrode.

1 ml of *0.05 M potassium iodate* is equivalent to 0.009832 g of $C_8H_8N_4,HCl$.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states that solutions containing glucose should not be used in the preparation of an intravenous infusion.

Hydrochloric Acid

Concentrated Hydrochloric Acid

HCl Mol. Wt. 36.5

Hydrochloric Acid contains not less than 35.0 per cent w/w and not more than 38.0 per cent w/w of HCl.

Category. Pharmaceutical aid (acidifying agent).

Description. A clear, colourless, fuming liquid.

Identification

A. When added to *potassium permanganate*, chlorine is evolved.

B. It gives the reactions of chlorides (2.3.1).

Tests

Arsenic (2.3.10). Mix 10 g with 40 ml of *water* and 1 ml of *stannous chloride solution AsT*; the resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). Evaporate 3.5 ml to dryness on a water-bath, add 2 ml of *dilute acetic acid* to the residue and add *water* to make 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

Bromide and iodide. Dilute 5 ml with 10 ml of *water*, add 1 ml of *chloroform* and, dropwise with constant shaking, *chlorinated lime solution*; the chloroform layer does not become brown or violet.

Free chlorine. To 15 ml, add 100 ml of *carbon dioxide-free water*, 1 ml of a 10 per cent w/v solution of *potassium iodide* and 0.5 ml of *starch solution* and allow to stand in the dark for 2 minutes. Any blue colour produced disappears on the addition of 0.2 ml of *0.01 M sodium thiosulphate*.

Sulphite. Dilute 1.0 ml with 10 ml of *water* and add 0.25 ml of *barium chloride solution* and 1 ml of *0.01 M iodine*; the colour of the iodine is not completely discharged.

Sulphates (2.3.17). Mix 6.5 ml with 10 mg of *sodium bicarbonate*, evaporate to dryness on a water-bath and dissolve the residue in 15 ml of *distilled water*. The resulting solution complies with the limit test for sulphates (20 ppm).

Residue on evaporation. Not more than 0.01 per cent, determined on 100 g by evporating to dryness on a water-bath and drying at 105°.

Assay. Weigh 2.0 g, add 30 ml of *water*, mix and titrate with *1 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.03646 g of HCl.

Storage. Store in stoppered containers of glass or any other inert material at a temperature not exceeding 30°.

Dilute Hydrochloric Acid

Dilute Hydrochloric Acid is prepared by mixing 274 g of Hydrochloric Acid and 726 g of Purified Water.

Dilute Hydrochloric Acid contains not less than 9.5 per cent and not more than 10.5 per cent w/w of HCl.

Description. A clear, colourless liquid.

Identification

A. When added to *potassium permanganate*, chlorine is evolved.

B. It gives the reactions of chlorides (2.3.1).

Tests

Arsenic (2.3.10). Mix 20.0 g with 20 ml of *water* and 1 ml of *stannous chloride AsT*; the resulting solution complies with the limit test for arsenic (0.5 ppm).

Heavy metals (2.3.13). Dissolve the residue obtained in the test for Residue on evaporation in 1 ml of *2 M hydrochloric acid*, dilute to 25.0 ml with *water;* to 2.5 ml of the resulting solution add 2 ml of *dilute acetic acid* and add *water* to make 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (2 ppm).

Free chlorine. To 60 ml add 50 ml of *carbon dioxide-free water*, 1 ml of a 10 per cent w/v solution of *potassium iodide* and 0.5 ml of *starch solution* and allow to stand in the dark for 2 minutes. Any blue colour produced disappears on the addition of 0.2 ml of *0.01 M sodium thiosulphate*.

Residue on evaporation. Not more than 0.01 per cent, determined on 100 g by evaporating to dryness on a water-bath and dry at 105°.

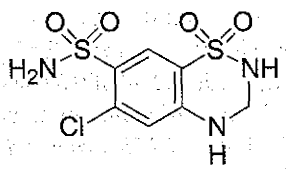
Sulphates (2.3.17). Mix 26 ml with 10 mg of *sodium bicarbonate*, evaporate to dryness on a water-bath and dissolve the residue in 15 ml of *distilled water*. The resulting solution complies with the limit test for sulphates (5 ppm).

Assay. Weigh 6.0 g, add 30 ml of *water*, mix and titrate with *1 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.03646 g of HCl.

Storage. Store in stoppered containers of glass or any other inert material at a temperature not exceeding 30°.

Hydrochlorothiazide



$C_7H_8ClN_3O_4S_2$ Mol. Wt. 297.7

Hydrochlorothiazide is 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide.

Hydrochlorothiazide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_8ClN_3O_4S_2$, calculated on the dried basis.

Category. Diuretic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydrochlorothiazide IPRS* or with the reference spectrum of hydrochlorothiazide.

B. When examined in the range 230 nm to 300 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum only at about 273 nm; absorbance at about 273 nm, 0.5 to 0.54. When examined in the range 300 nm to 360 nm, a 0.005 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum at 323 nm; absorbance at about 323 nm, 0.45 to 0.48.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Ethyl acetate.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of *acetone*.

Reference solution. A 0.5 per cent w/v solution of *hydrochlorothiazide IPRS* in *acetone*.

Apply to the plate 4 µl of each solution. After development, dry the plate in a current of air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Heat gently about 1 mg with 2 ml of a freshly prepared 0.05 per cent w/v solution of *chromotropic acid sodium salt* in a cooled mixture of 7 volumes of *water* and 13 volumes of *sulphuric acid;* a violet colour develops.

Tests

Acidity or alkalinity. Shake 0.5 g of the powdered substance under examination with 25 ml of *water* for 2 minutes and filter. To 10 ml of the filtrate add 0.2 ml of *0.01 M sodium hydroxide* and 0.15 ml of *methyl red solution*. The solution is yellow and not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 35.8 g of *disodium hydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.2 with *dilute phosphoric acid*. Dilute 100.0 ml of the solution to 2000.0 ml with *water*.

Solvent mixture (a). A mixture of equal volumes of *acetonitrile* and *methanol*.

Solvent mixture (b). Dilute 50.0 ml of solvent mixture (a) to 200.0 ml with *buffer solution*.

Test solution. Dissolve 30 mg of the substance under examination in 5 ml of solvent mixture (a) with the aid of ultrasound and dilute to 20.0 ml with *buffer solution*.

Reference solution (a). Dissolve 15 mg, each of, *hydrochlorothiazide IPRS* and *hydrochlorothiazide impurity A IPRS (chlorothiazide IPRS)* in 25 ml of solvent mixture (a) and dilute to 100 ml with *buffer solution*. Dilute 5.0 ml of the solution to 100.0 ml with solvent mixture (b).

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with solvent mixture (b). Dilute 5.0 ml of the solution to 20.0 ml with solvent mixture (b).

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. to 940 ml of *buffer solution*, add 60.0 ml of *methanol* and 10.0 ml of *tetrahydrofuran*,
  B. to a mixture of 500 ml of *methanol* and 500 ml of *buffer solution*, add 50.0 ml of *tetrahydrofuran*,
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 224 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
17	55	45
30	55	45
35	100	0
50	100	0

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to hydrochlorothiazide impurity A and hydrochlorothiazide is not less than 2.5.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (2.3.12). Dissolve 1.0 g in 25 ml of *acetone* and dilute to 30 ml with *water*. The resulting solution complies with the limit test for chlorides (250 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances with the following modification.

Test solution (a). Dilute 1.0 ml of test solution to 20.0 ml with *buffer solution*.

Reference solution(c). Dissolve 30.0 mg of *hydrochlorothiazide IPRS* in 5 ml of a solvent mixture (a), using sonication if necessary, and dilute to 20.0 ml with *buffer solution*. Dilute 1.0 ml of the solution to 20.0 ml with *buffer solution*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane silica gel (3 µm),
- mobile phase: A. to 940 ml of *buffer solution*, add 60.0 ml of *methanol* and 10.0 ml of *tetrahydrofuran* and mix,
  B. to a mixture of 500 ml of *methanol* and 500 ml of *buffer solution*, add 50.0 ml of *tetrahydrofuran* and mix,
- a gradient programme using the conditions given below,
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 224 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
4	80	20
10	20	80
11	80	20

Name	Relative retention time
Impurity A[1]	0.9
Hydrochlorothiazide (Retention time: about 2.2 minutes)	1.0

[1]6-chloro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (chlorothiazide).

Inject the reference solution (a). The test is not valid unless the resolution between the peaks due to impurity A and hydrochlorothiazide is not less than 2.

Inject the reference solution (c) and the test solution (a).

Calculate the content of $C_7H_8ClN_3O_4S_2$.

Hydrochlorothiazide Tablets

Hydrochlorothiazide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

Usual strengths. 25 mg; 50 mg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Ethyl acetate.

Test solution. Triturate a quantity of the powdered tablets containing 10 mg of Hydrochlorothiazide with 10 ml of *acetone* and filter.

Reference solution. A 0.1 per cent w/v solution of *hydrochlorothiazide IPRS* in *acetone*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of air, examine under ultraviolet light at 254 nm and then spray with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed tank for 15 minutes (the nitrous fumes may be generated by adding *7 M sulphuric acid* dropwise to a solution containing 10 per cent w/v of *sodium nitrite* and 3 per cent w/v of *potassium iodide*). Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)*. Examine the plate again. By each method of visualisation the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the solution and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 mm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 272 nm (2.4.7). Calculate the content of $C_7H_8ClN_3O_4S_2$ taking 644 as the specific absorbance at 272 nm.

Q. Not less than 60 per cent of the stated amount of $C_7H_8ClN_3O_4S_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 35.8 g of *disodium hydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.2 with *dilute phosphoric acid*. Dilute 100.0 ml of the solution to 2000.0 ml with *water*.

Solvent mixture. A mixture of equal volumes of *acetonitrile* and *methanol*.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Hydrochlorothiazide with 25 ml of the solvent mixture and dilute to 100.0 ml with buffer solution, filter through a glass fibre filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with a mixture containing 1 volume of *methanol*, 1 volume of *acetonitrile* and 2 volumes of buffer solution.

Reference solution (b). Dissolve 15 mg, each of, *hydrochlorothiazide IPRS* and *hydrochlorothiazide impurity A IPRS (chlorothiazide IPRS)* in 25 ml of the solvent mixture and dilute to 100.0 ml with buffer solution. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (Such as Phenosphere ODS or Microsorb ODS),
- mobile phase: A. a mixture of 10 volumes of *tetrahydrofuran*, 60 volumes of *methanol* and 940 volumes of *buffer solution*,

  B: a mixture of 50 volumes of *tetrahydrofuran*, 500 volumes of *methanol* and 500 volumes of buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 224 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
17	55	45
30	55	45
35	100	0
50	100	0

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to chlorothiazide and hydrochlorothiazide is not less than 2.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). The sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 30 mg of hydrochlorothiazide in 20 ml of the mobile phase with the aid of ultrasound, add 20 ml of *acetonitrile* and further sonicate for 5 minutes, with intermittent shaking and dilute to 200.0 ml with the mobile phase, filter.

Reference solution (a). A 0.015 per cent w/v solution of *hydrochlorothiazide IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.015 per cent w/v, each of, *hydrochlorothiazide IPRS* and *chlorothiazide IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of *0.1M monobasic sodium phosphate* and 10 volumes of *acetonitrile*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The relative retention time for chlorothiazide with respect to hydrochlorothiazide is about 0.8.

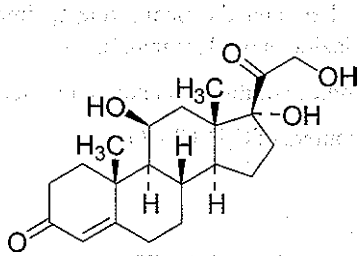
Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to hydrochlorothiazide and chlorothiazide is not less than 2.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 1.5 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_7H_8ClN_3O_4S_2$ in the tablets.

Hydrocortisone

Cortisol



$C_{21}H_{30}O_5$ Mol. Wt. 362.5

Hydrocortisone is 11β,17α ,21-trihydroxypregn-4-ene-3, 20- dione.

Hydrocortisone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{21}H_{30}O_5$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white to practically white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydrocortisone IPRS* or with the reference spectrum of hydrocortisone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase (a). A mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water*.

Mobile phase (b). A mixture of 80 volumes of *ether*, 15 volumes of *toluene* and 5 volumes of *1-butanol* saturated with *water*.

Solvent mixture. 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Dissolve 0.25 g of the substance under examination in 100.0 ml with solvent mixture.

Reference solution. A 0.25 per cent w/v solution of *hydrocortisone IPRS* in the same solvent mixture.

Apply to the plate 2 µl of each solution. Develop the chromatograms successively with each mobile phase. After both developments, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Spray the plate with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes or until spots appear and allow to cool. Examine the chromatograms in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution is similar in colour in daylight, fluorescence under ultraviolet light at 365 nm, position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To 2 ml of a 0.1 per cent w/v solution in *ethanol (95 per cent)* add 2 ml of *sulphuric acid*; an intense yellow colour is produced with a green fluorescence, which is particularly intense under ultraviolet light at 365 nm. Add the solution to 10 ml of *water* and mix; the fluorescence under ultraviolet light at 365 nm does not disappear.

Tests

Specific optical rotation (2.4.22). +150° to +156°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). A 0.001 per cent w/v solution in *ethanol (95 per cent)* exhibits a maximum at about 240 nm; absorbance at about 240 nm, between 0.42 and 0.45.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in sufficient *tetrahydrofuran* to produce 10 ml.

Reference solution (a). Dissolve 2 mg of *hydrocortisone IPRS* and 2 mg of *prednisolone IPRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 220 ml of *tetrahydrofuran* and 700 ml of *water*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Inject reference solution (a). The retention times are: prednisolone, about 14 minutes and hydrocortisone about 15.5 minutes. The test is not valid unless the resolution between the peaks corresponding to prednisolone and hydrocortisone is at least 2.2. If necessary, adjust the concentration of *tetrahydrofuran* in the mobile phase.

Inject reference solution (b) and the test solution. Run the chromatogram of the test solution for 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent): the sum of the areas of all the peaks other than the principal peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh 0.1 g, dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at 241.5 nm. Calculate the content of $C_{21}H_{30}O_5$ taking 440 as the specific absorbance at 241.5 nm.

Storage. Store protected from light.

Hydrocortisone Ointment

Hydrocortisone Ointment contains Hydrocortisone in a suitable base.

Hydrocortisone Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hydrocortisone, $C_{21}H_{30}O_5$.

Usual strengths. 0.5 per cent w/w; 1 per cent w/w; 2.5 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *octadecylsilane silica gel G*.

For ointments containing more than 0.5 per cent w/w of Hydrocortisone—

Test solution. Disperse a quantity of ointment containing 25 mg of Hydrocortisone in 5 ml of hot *hexane*, cool, extract with 10 ml of *methanol (90 per cent)* and filter.

Reference solution (a). A 0.25 per cent w/v solution of *hydrocortisone IPRS* in *methanol*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

For ointments containing 0.5 per cent w/w or less of Hydrocortisone—

Test solution. Disperse a quantity of ointment containing 5 mg of Hydrocortisone in 50 ml of hot *hexane*, cool, extract with 10 ml of *methanol (90 per cent)* and filter.

Reference solution (a). A 0.05 per cent w/v solution of *hydrocortisone IPRS* in *methanol*.

Reference solution (b). A mixture of equal volumes of the reference solution (a) and the test solution.

Mobile phase. A mixture of 1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with *alkaline tetrazolium blue solution* and examine in day light. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). If it does not, the principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. In the Assay, the chromatogram obtained with the test solution (b) corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Ointment.

Assay. Determine by liquid chromatography (2.4.14).

For ointment containing more than 0.5 per cent w/w of Hydrocortisone—

Solution A. A 0.5 per cent w/v solution of *betamethasone* (internal standard) in *methanol*.

Test solution (a). Disperse a quantity of ointment containing 25 mg of Hydrocortisone in 40 ml of a mixture of 3 volumes of *methanol* and 1 volume of a 15 per cent w/v solution of *sodium chloride* by shaking. Add 50 ml of hot *hexane*, shake and separate the lower layer. Repeat the extraction using a further two 10-ml quantities of the *methanolic sodium chloride solution*. To the combined extracts add 5 ml of solution A and dilute to 100.0 ml with *water*, filter through glass mimcrofibre filter paper.

Test solution (b). Disperse a quantity of ointment containing 25 mg of Hydrocortisone in 100 ml of hot *hexane*, cool and extract with 20 ml of a solution prepared by mixing 3 volumes of *methanol* with 1 volume of a 15 per cent w/v solution of *sodium chloride*. Repeat the extraction using a further two 10-ml quantities of the *methanolic sodium chloride solution*. To the combined extracts add 5 ml of *methanol* and dilute to 100.0 ml with *water*, filter through glass microfibre filter paper.

Reference solution. Dissolve 25 mg of *hydrocortisone IPRS* in 45 ml of *methanol*, add 5 ml of solution A and dilute to 100.0 ml with *water*.

For ointments containing 0.5 per cent w/w or less of Hydrocortisone—

Solution B. A 0.11 per cent w/v solution of *betamethasone* (internal standard) in *methanol*.

Test solution (a). Disperse a quantity of the ointment containing 5 mg of Hydrocortisone in 40 ml of a mixture of 3 volumes of *methanol* and 1 volume of a 15.0 per cent w/v solution of *sodium chloride*. Add 50 ml of hot *hexane*, shake and separate the lower layer. Repeat the extraction using a further two 10-ml quantities of the *methanolic sodium chloride solution*. To the combined extracts add 5 ml of solution B and dilute to 100.0 ml with *water*, filter.

Test solution (b). Disperse a quantity of ointment containing 5 mg of Hydrocortisone in 100 ml of hot *hexane*, cool and extract with 20 ml of a solution prepared by mixing 3 volumes of *methanol* with 1 volume of a 15 per cent w/v solution of *sodium chloride*. Repeat the extraction using a further two 10-ml quantities of the *methanolic sodium chloride solution*. To the combined extracts add 5 ml of *methanol* and dilute to 100 ml with *water*, filter.

Reference solution. Dissolve 5 mg of *hydrocortisone IPRS* in 45 ml of *methanol* and add 5 ml of solution B and dilute to 100.0 ml with *water*.

Chromatographic system
- a stainless steel column 10 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 50 per cent v/v *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

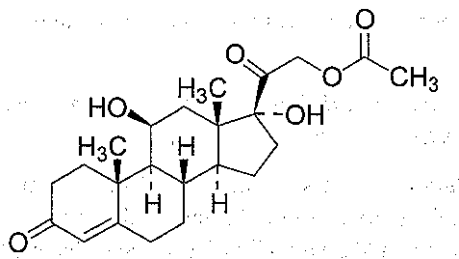
Inject the reference solution and test solution (a).

Calculate the content of $C_{21}H_{30}O_5$ in the ointment.

Storage. Store protected from light.

Hydrocortisone Acetate

Cortisol Acetate



$C_{23}H_{32}O_6$ Mol. Wt. 404.5

Hydrocortisone Acetate is 11β,17α-dihydroxy-3,20- dioxopregn-4-en-21-yl acetate.

Hydrocortisone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{23}H_{32}O_6$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C, and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydrocortisone acetate IPRS* or with the reference spectrum of hydrocortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Add a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

Test solution (a). Dissolve 25 mg of the substance under examination in *methanol* and dilute to 5 ml with the same solvent. Use this solution to prepare test solution (b). Dilute 2.0 ml of the solution to 10.0 ml with *dichloromethane*.

Test solution (b). Transfer 2.0 ml of the solution obtained in preparing test solution (a) to a 15-ml glass tube with a glass or plastic stopper. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution* and immediately pass a stream of *nitrogen* through the solution for 5 minutes. Stopper the tube. Heat in a water-bath at 45° protected from light for 2½ hours. Allow to cool.

Reference solution (a). Prepare in the same manner as test solution (a) but using *hydrocortisone acetate IPRS* in place of the substance under examination.

Reference solution (b). Prepare in the same manner as test solution (b) but using 2 ml of the solution obtained in preparing reference solution (a) in place of the solution obtained in preparing test solution (a).

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with *ethanolic sulphuric acid (20 per cent v/v)* and heat at 120° for 10 minutes or until the spots appear. Allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence under ultraviolet light at 365 nm, position and size to that in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an R_f value distinctly lower than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

C. To 2 ml of a 0.1 per cent w/v solution in *ethanol (95 per cent)* add 2 ml of *sulphuric acid* and mix; an orange colour is produced with a green fluorescence which is particularly intense under ultraviolet light at 365 nm. Add the solution to 10 ml of *water* and mix; the fluorescence under ultraviolet light at 365 nm does not disappear.

D. It gives the reaction of acetyl groups (2.3.1).

Tests

Specific optical rotation (2.4.22). +158° to +167°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). A 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 240 nm; absorbance at about 240 nm, between 0.38 and 0.40.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in sufficient *methanol* to produce 10 ml.

Reference solution (a). Dissolve 2 mg of *hydrocortisone acetate IPRS* and 2 mg of *cortisone acetate IPRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 400 ml of *acetonitrile* and 550 ml of *water*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Inject reference solution (a). The retention times are: hydrocortisone acetate, about 10 minutes and cortisone acetate about 12 minutes. The test is not valid unless the resolution between the peaks corresponding to hydrocortisone acetate and cortisone acetate is at least 4.2. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject reference solution (b) and the test solution. Continue the chromatography of the test solution for 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than one such peak has an area greater than half the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 2.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh 0.1 g, dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at 241.5 nm. Calculate the content of $C_{23}H_{32}O_6$ taking 395 as the specific absorbance at 241.5 nm.

Storage. Store protected from light.

Hydrocortisone Cream

Hydrocortisone Acetate Cream; Cortisol Acetate Cream

Hydrocortisone Cream contains Hydrocortisone Acetate in a suitable cream base.

Hydrocortisone Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hydrocortisone acetate, $C_{23}H_{32}O_6$.

Usual strengths. 1 per cent; 2.5 per cent.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water*.

For creams containing more than 0.5 per cent w/w of Hydrocortisone Acetate—

Test solution. Disperse a quantity of the cream containing 25 mg of Hydrocortisone Acetate with 10 ml of *methanol (90 per cent)*, add 50 ml of hot *hexane* and shake. Discard the upper layer, add 5 g of *anhydrous sodium sulphate* to the lower layer, mix and filter through a glass microfiber filter paper.

Reference solution. A mixture of equal volumes of the test solution and a 0.25 per cent w/v solution of *hydrocortisone acetate IPRS* in *methanol*.

For creams containing 0.5 per cent w/w or less of Hydrocortisone Acetate—

Test solution. Disperse a quantity of the cream containing 5 mg of Hydrocortisone Acetate with 10 ml of *methanol (90 per cent)*, add 50 ml of hot *hexane* and shake. Discard the upper layer, add 5 g of *anhydrous sodium sulphate* to the lower layer, mix and filter through a glass microfiber filter paper.

Reference solution. A mixture of equal volumes of the test solution and a 0.05 per cent w/v solution of *hydrocortisone acetate IPRS* in *methanol*.

Apply to the plate 5 µl of each solution. After development dry the plate in air and spray with *alkaline tetrazolium blue solution* and examine in day light. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution, which appears as a single, compact spot.

B. In the Assay, the chromatogram obtained with test solution (a) corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

For creams containing more than 0.5 per cent w/w of Hydrocortisone Acetate—

Test solution (a). Disperse a quantity containing 25 mg of Hydrocortisone Acetate in 40 ml of a solution prepared by mixing 75 ml of *methanol* with 25 ml of a 15 per cent w/v solution of *sodium chloride* by shaking. Add 50 ml of hot *hexane*, shake and separate the lower layer. Repeat the extraction using two 10-ml quantities of the *methanolic sodium chloride solution*. Add 5 ml of *methanol* to the combined extracts and dilute to 100.0 ml with *water*, mix and filter through a glass microfiber filter paper.

Test solution (b). Prepare in the same manner as test solution (a) but adding 5 ml of internal standard solution in place of the 5 ml of *methanol* before diluting to 100.0 ml.

Reference solution. Dissolve 25 mg of *hydrocortisone acetate IPRS* in 45 ml of *methanol*, add 5 ml of a 0.5 per cent w/v solution of *betamethasone* (internal standard) in *methanol* and dilute to 100.0 ml with *water*.

For creams containing 0.5 per cent w/w or less of Hydrocortisone Acetate—

Test solution (a). Disperse a quantity containing 5 mg of Hydrocortisone Acetate in 40 ml of a solution prepared by mixing 75 ml of *methanol* with 25 ml of a 15 per cent w/v solution of *sodium chloride* by shaking. Add 50 ml of hot *hexane*, shake and separate the lower layer. Repeat the extraction using two 10-ml quantities of the *methanolic sodium chloride solution*. Add 5 ml of *methanol* to the combined extracts and dilute to 100.0 ml with *water*, mix and filter through a glass microfiber filter paper.

Test solution (b). Prepare in the same manner as test solution (a) but adding 5 ml of internal standard solution in place of the 5 ml of *methanol* before diluting to 100.0 ml.

Reference solution. Dissolve 5 mg of *hydrocortisone acetate IPRS* in 45 ml of *methanol*, add 5 ml of a 0.11 per cent w/v solution of *betamethasone* (internal standard) in *methanol* and dilute to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 10 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 50 per cent v/v *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution and test solution (b).

Calculate the content of $C_{23}H_{32}O_6$ in the Cream.

Hydrocortisone Eye Ointment

Hydrocortisone Acetate Eye Ointment; Cortisol Acetate Eye Ointment

Hydrocortisone Eye Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hydrocortisone acetate, $C_{23}H_{32}O_6$.

Usual strength. 2.5 per cent w/w.

Identification

Boil 2 g with 20 ml of *methanol*, shake, cool to 0° for 30 minutes, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

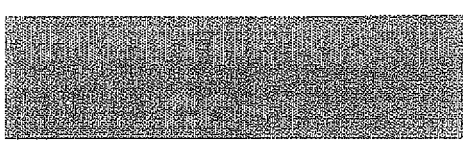
Reference solution (a). Dissolve 25 mg of *hydrocortisone IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. Dissolve about 1 mg in 1 ml of *sulphuric acid* and mix; an orange colour is produced with a green fluorescence, which is particularly intense under ultraviolet light at 365 nm. Add the solution to 10 ml of *water* and mix; the fluorescence under ultraviolet light at 365 nm does not disappear.



C. Dissolve 10 mg in 1 ml of *methanol*, warm and add 1 ml of *potassium cupri-tartrate solution*; an orange-red precipitate is slowly formed.

Tests

Other tests. Comply with the tests stated under Eye Ointments.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the ointment containing 10 mg of Hydrocortisone Acetate, shake with 20.0 ml of *methanol* for 30 minutes and centrifuge. To 10.0 ml of the clear, supernatant layer add sufficient *methanol* to produce 50.0 ml.

Reference solution. A solution containing 0.01 per cent w/v of *hydrocortisone acetate IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with porous silica particles (5 µm),
- mobile phase: a mixture of 425 volumes of *butyl chloride* saturated with *water*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol* and 30 volumes of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Calculate the content of $C_{23}H_{32}O_6$ in the ointment.

Storage. Store protected from light at a temperature not exceeding 30°.

Hydrocortisone Acetate Injection

Cortisol Acetate Injection

Hydrocortisone Acetate Injection is a sterile suspension of a very fine powder of Hydrocortisone Acetate in Water for Injections or Sodium Chloride Injection containing suitable dispersing agents.

Hydrocortisone Acetate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of hydrocortisone acetate, $C_{23}H_{32}O_6$.

Usual strength. 25 mg per ml.

Identification

Filter a volume containing 50 mg of Hydrocortisone Acetate through a sintered-glass filter, wash the residue with four quantities, each of 5 ml, of *water*, dissolve in 20 ml of *chloroform*, wash the chloroform solution with four quantities, each of 10 ml, of *water*, discard the washings, filter the chloroform solution through a plug of cotton and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydrocortisone acetate IPRS* or with the reference spectrum of hydrocortisone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *hydrocortisone acetate IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Other tests. Comply with the tests stated under Parenteral Preparations (Injections)

Assay. Determine by liquid chromatography (2.4.14).

Test solution. To a measured volume of the injection containing about 50 mg of Hydrocortisone Acetate add 70 ml of *methanol*, shake to produce a clear solution and dilute to 100.0 ml with *methanol*. Dilute 10.0 ml of the resulting solution to 20.0 ml with *water*.

Reference solution. Dissolve 25.0 mg of *hydrocortisone acetate IPRS* in 50 ml of *methanol* and add sufficient *water* to produce 100.0 ml.

Chromatographic system
- a stainless steel column 10 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 1),

- mobile phase: 50 per cent v/v solution of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

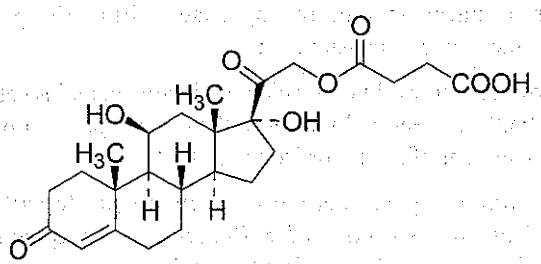
Calculate the content of $C_{23}H_{32}O_6$ in the injection.

Storage. Store protected from light.

Labelling. The label states (1) that the contents are meant for local injection only; (2) that the container should be gently shaken before a dose is withdrawn; (3) the names of the dispersing agents used, if any.

Hydrocortisone Hemisuccinate

Hydrocortisone Hydrogen Succinate; Cortisol Hydrogen Succinate



$C_{25}H_{34}O_8$ Mol. Wt. 462.6

Hydrocortisone Hemisuccinate is 11β,17α -dihydroxy-3,20-dioxopregn-4-en-21-yl hydrogen succinate.

Hydrocortisone Hemisuccinate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{25}H_{34}O_8$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white or almost white, crystalline powder; hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Dry the substances before use at 105° for 3 hours and examine them as discs. Compare the spectrum with that obtained with *hydrocortisone hemisuccinate IPRS* or with the reference spectrum of hydrocortisone hemisuccinate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Add a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

Test solution (a). Dissolve 25 mg of the substance under examination in *methanol* and dilute to 5 ml with the same solvent. Use this solution to prepare test solution (b). Dilute 2.0 ml of the solution to 10.0 ml with *dichloromethane*.

Test solution (b). Transfer 2.0 ml of the solution obtained in preparing test solution (a) to a 15-ml glass tube with a glass or plastic stopper. Add 10 ml of a 0.8 g per litre solution of *sodium hydroxide* in *methanol* and immediately pass a stream of *nitrogen* through the solution for 5 minutes. Stopper the tube. Heat in a water-bath at 45° protected from light for 30 minutes. Allow to cool.

Reference solution (a). Prepare in the same manner as test solution (a) but using *hydrocortisone hemisuccinate IPRS* in place of the substance under examination.

Reference solution (b). Prepare in the same manner as test solution (b) but using 2 ml of the solution obtained in preparing reference solution (a) in place of the solution obtained in preparing test solution (a).

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with *ethanolic sulphuric acid (20 per cent v/v)* and heat at 120° for 10 minutes or until the spots appear. Allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence under ultraviolet light at 365 nm, position and size to that in the chromatogram obtained with the corresponding reference solution. The principal spots in the chromatograms obtained with test solution (b) and reference solution (b) have an R_f value distinctly higher than that of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a).

C. Add about 2 mg to 2 ml of *sulphuric acid;* a yellow to brownish red colour develops with a green fluorescence that is particularly intense when examined under ultraviolet light at 365 nm.

D. About 30 mg gives the reaction of esters (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *sodium bicarbonate solution* is clear (2.4.1).

Light absorption (2.4.7). A 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 240 nm; absorbance at about 240 nm, between 0.34 and 0.36.

Specific optical rotation (2.4.22). +147° to +153°, determined in a 1.0 per cent w/v solution in *ethanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in sufficient *methanol* to produce 10 ml.

Reference solution (a). Dissolve 2 mg of *hydrocortisone hemisuccinate IPRS* and 2 mg of *dexamethasone IPRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 330 ml of *acetonitrile* and 600 ml of *water* and 1.0 ml of *orthophosphoric acid*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Inject reference solution (a). The retention times are: dexamethasone, about 12.5 minutes and hydrocortisone hemisuccinate about 15 minutes. The test is not valid unless the resolution between the peaks corresponding to dexamethasone and hydrocortisone hemisuccinate is at least 5.0. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject reference solution (b) and the test solution. Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak other than the principal peak, is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.1 g, dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol* and mix. Measure the absorbance of the resulting solution (2.4.7) at the maximum at about 241.5 nm. Calculate the content of $C_{25}H_{34}O_8$ taking 353 as the specific absorbance at 241.5 nm.

Storage. Store protected from light and moisture.

Hydrocortisone Sodium Succinate Injection

Cortisol Sodium Succinate Injection

Hydrocortisone Sodium Succinate Injection is a sterile material made from Hydrocortisone Hemisuccinate with the aid of a suitable alkali such as Sodium Hydroxide or Sodium Carbonate. It may contain suitable buffering agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Hydrocortisone Sodium Succinate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of hydrocortisone, $C_{21}H_{30}O_5$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strengths. The equivalent of 100 mg and 500 mg of hydrocortisone.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with the reference spectrum of *hydrocortisone sodium succinate IPRS* or with the reference spectrum of hydrocortisone sodium succinate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A freshly prepared mixture of 1 volume of *anhydrous formic acid*, 10 volumes of *absolute ethanol* and 150 volumes of *dichloromethane*.

Test solution. A 0.1 per cent w/v solution of the contents of the sealed container in a mixture of 1 volume of *methanol* and 9 volumes of *dichloromethane*.

Reference solution (a). A 0.1 per cent w/v solution of *hydrocortisone sodium succinate IPRS* in the same solvent mixture.

Reference solution (b). A solution containing 0.1 per cent w/v, each of, *hydrocortisone hemisuccinate IPRS* and *methylprednisolone hydrogen succinate IPRS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the solvents have evaporated, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. By each method of visualization the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two spots that may not be completely separated.

Tests

pH (2.4.24). 6.5 to 8.0, determined in a solution containing the equivalent of 5.0 per cent w/v of hydrocortisone.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of equal volumes of *acetonitrile* and *water*.

Test solution. Dissolve a sufficient quantity of the contents of the sealed container in a solvent mixture to produce a solution containing the equivalent of 0.25 per cent w/v of hydrocortisone.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with solvent mixture.

Reference solution (b). Dilute 0.035 per cent w/v solution of *hydrocortisone IPRS* in *acetonitrile* with solvent mixture.

Reference solution (c). Dilute a solution containing 0.04 per cent w/v, each of, *hydrocortisone hemisuccinate IPRS* and *dexamethasone IPRS* with solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 330 ml of *acetonitrile* and 600 ml of *water* and 1 ml of *orthophosphoric acid*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Inject reference solution (c). The test is not valid unless in the chromatogram obtained the resolution between the peaks corresponding to dexamethasone and hydrocortisone hemisuccinate is at least 5.0.

Inject the test solution. Allow the chromatography to proceed for twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to hydrocortisone is not greater than the area of the peak in the chromatogram obtained with reference solution (b) (7 per cent) and the area of any other secondary peak is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (2 per cent).

Bacterial endotoxins (2.2.3). Not more than 1.25 Endotoxin Units per mg of Hydrocortisone.

Assay. Determine the weight of the contents of 10 containers. Dissolve the mixed contents of the 10 containers in sufficient *water* to produce a solution containing the equivalent of 0.001 per cent w/v of hydrocortisone. Measure the absorbance of the resulting solution at the maximum at about 248 nm (2.4.7). Calculate the content of $C_{21}H_{30}O_5$ taking 449 as the specific absorbance at 248 nm.

Storage. Store protected from moisture in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the strength in terms of the equivalent amount of hydrocortisone; (2) that the prepared solution should be used only if it is clear; (3) that the solution should be used immediately after preparation.

Hydrogenated Vegetable Oil

Hydrogenated Vegetable Oil is a mixture of triglycerides of fatty acids of vegetable origin.

Category. Pharmaceutical aid.

Description. An almost white, fine powder at room temperature and a pale yellow, oily liquid above its melting point.

Identification

Comply with the tests for Acid value (2.3.23), Iodine value (2.3.28) and Saponification value (2.3.37).

Tests

Melting range (2.4.21). 57° to 85°.

Acid value (2.3.23). Not more than 4.0.

Iodine value (2.3.28). Not more than 5.

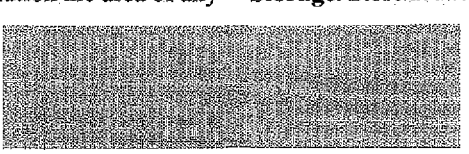
Saponification value (2.3.37). 175 to 200.

Unsaponifiable matter (2.3.39). Not more than 0.8 per cent w/w.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Loss on drying (2.4.19). Not more than 0.1 per cent, determined on 5.0 g by drying in oven at 105° for 4 hours.

Storage. Store at a temperature between 8° to 25°.



Hydrogen Peroxide Solution (20 Vol)

Hydrogen Peroxide Solution (6 per cent); Dilute Hydrogen Peroxide Solution

H_2O_2 Mol. Wt. 34.0

Hydrogen Peroxide Solution (20 Vol) is an aqueous solution of hydrogen peroxide. It may contain a suitable stabilising agent.

Hydrogen Peroxide Solution (20 Vol) contains not less than 5.0 per cent w/v and not more than 7.0 per cent w/v of H_2O_2, corresponding to about 20 times its volume of available oxygen.

Category. Antiseptic; deodorant.

Description. A clear, colourless liquid. It decomposes in contact with oxidisable organic matter and with certain metals and also if allowed to become alkaline.

Identification

A. To 1 ml add 0.2 ml of *1 M sulphuric acid* and 0.25 ml of *0.02 M potassium permanganate*; the solution becomes colourless with evolution of gas.

B. Shake 0.05 ml with 2 ml of *1 M sulphuric acid*, 2 ml of *ether* and 0.05 ml of *potassium chromate solution*; the ether layer becomes blue.

Tests

Acidity. To 10 ml add 20 ml of *water* and 0.25 ml of *methyl red solution*. Not less than 0.2 ml and not more than 1.0 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Organic stabilizers. Shake 20 ml with successive quantities of 10, 5 and 5 ml of *chloroform*. Evaporate the combined chloroform extracts at a temperature not exceeding 25° at a pressure of 2 kPa and dry in a desiccator. The residue weighs not more than 10 mg (500 ppm).

Non-volatile matter. Place 10 ml in a platinum dish and allow to stand until effervescence ceases. Evaporate the solution on a water-bath and dry the residue at 105°; the residue weighs not more than 20 mg.

Assay. To 1.0 ml add 20 ml of *1 M sulphuric acid* and titrate with *0.02 M potassium permanganate*.

1 ml of *0.02 M potassium permanganate* is equivalent to 0.001701 g of H_2O_2 or 0.56 ml of oxygen.

Storage. Store protected from light in containers resistant to hydrogen peroxide at a temperature not exceeding 30°. If the solution does not contain a stabilising agent, it should be stored in a refrigerator (2° to 8°). It should not be stored for long periods.

Labelling. The label states whether or not the solution contains a stabilising agent.

Hydrogen Peroxide Solution (100 Vol)

Hydrogen Peroxide Solution (27 per cent); Strong Hydrogen Peroxide Solution

Hydrogen Peroxide Solution (100 Vol) is an aqueous solution of hydrogen peroxide. It may contain a suitable stabilising agent.

Hydrogen Peroxide Solution (100 Vol) contains not less than 26.0 per cent w/w and not more than 28.0 per cent w/w of H_2O_2, corresponding to about 100 times its volume of available oxygen.

Category. Antiseptic; deodorant.

Description. A clear, colourless liquid. It decomposes vigorously in contact with oxidisable organic matter and with certain metals and also if allowed to become alkaline.

Identification

A. To 1 ml add 0.2 ml of *1 M sulphuric acid* and 0.25 ml of *0.02 M potassium permanganate*; the solution becomes colourless with evolution of gas.

B. Shake 0.05 ml with 2 ml of *1 M sulphuric acid*, 2 ml of *ether* and 0.05 ml of *potassium chromate solution*; the ether layer becomes blue.

Tests

Acidity. Dilute 10 ml with 100 ml of *water* and add 0.25 ml of *methyl red solution*. Not less than 0.05 ml and not more than 0.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Organic stabilisers. Shake 20 ml with successive quantities of 10, 5 and 5 ml of *chloroform*. Evaporate the combined chloroform extracts at a temperature not exceeding 25° at a pressure of 2 kPa and dry in a desiccator. The residue weighs not more than 10 mg (500 ppm).

Non-volatile matter. Place 10 ml in a platinum dish and allow to stand until effervescence ceases. Evaporate the solution on a water-bath and dry the residue at 105°; the residue weighs not more than 20 mg.

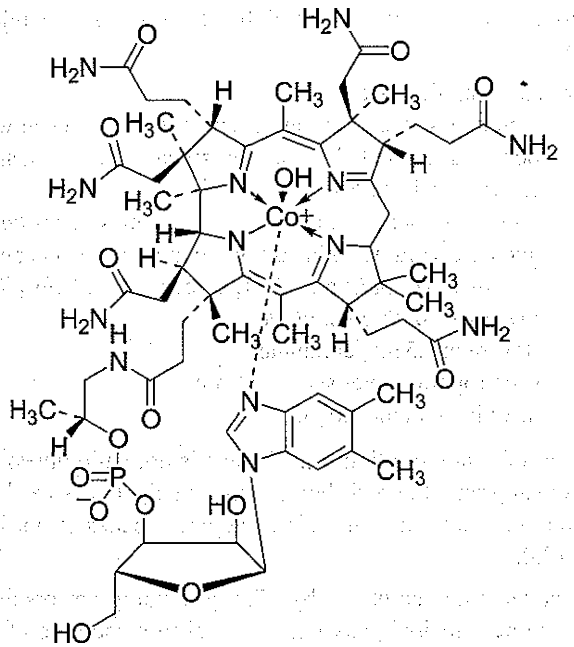
Assay. Dilute about 1.0 g to 100.0 ml with *water*. To 10.0 ml of the resulting solution add 20 ml of *1 M sulphuric acid* and titrate with *0.02 M potassium permanganate*.

1 ml of *0.02 M potassium permanganate* is equivalent to 0.001701 g of H_2O_2 or 0.56 ml of oxygen.

Storage. Store protected from light in containers resistant to hydrogen peroxide at a temperature not exceeding 30°. If the solution does not contain a stabilising agent, it should be stored in a refrigerator (2° to 8°). It should not be stored for long periods.

Labelling. The label states whether or not the solution contains a stabilising agent.

Hydroxocobalamin



$C_{62}H_{89}CoN_{13}O_{15}P$ Mol. Wt. 1346.4

Hydroxocobalamin is Coα-[α-(5,6-dimethylbenzimidazolyl)]-Coβ-hydroxocobamide. It occurs either as aquocobalamin chloride (Coα-[α-(5,6-dimethylbenzimidazolyl)]-ICoβ-aquocobamide chloride) or as aquocobalamin sulphate.

Hydroxocobalamin contains not less than 96.0 per cent and not more than 102.0 per cent of aquocobalamin chloride, $C_{62}H_{89}CoN_{13}O_{15}P,HCl$, or of aquocobalamin sulphate, $C_{124}H_{178}Co_2N_{26}O_{30}P_2,H_2SO_4$, both calculated on the dried basis.

Category. Vitamin B_{12} analogue used as haematopoietic.

Description. Dark red crystals or a crystalline powder; very hygroscopic. Some decomposition may occur on drying.

Identification

A. Measure the absorbance of the solution used in the Assay at the maxima at about 274 nm, 351 nm and 525 nm (2.4.7); ratios of the absorbances at about 274 nm and 525 nm to that at about 351 nm, about 0.8 and about 0.3 respectively.

B. Fuse 1 mg of the substance with 50 mg of *potassium hydrogen sulphate*, cool, break up the mass, add 3 ml of *water* and boil until dissolved. Add 0.05 ml of *phenolphthalein solution* and sufficient *5 M sodium hydroxide* to produce a faint pink colour. Add 0.5 g of *sodium acetate*, 0.5 ml of *1 M acetic acid* and 0.5 ml of a 0.2 per cent w/v solution of *nitroso R salt*; a red or orange-red colour is produced immediately. Add 0.5 ml of *hydrochloric acid* and boil for 1 minute; the red colour persists.

C. It gives the reactions of chlorides or of sulphates, as the case may be (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and protect them from bright light.

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dilute 5.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). Dissolve 25 mg of the substance under examination in 10 ml of *water*, warming if necessary. Allow to cool and add 1 ml of a 2.0 per cent w/v solution of *chloramine T* and 0.5 ml of *0.05 M hydrochloric acid*. Dilute to 25.0 ml with *water*. Shake and allow to stand for 5 minutes. Inject immediately.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 19.5 volumes of *methanol* and 80.5 volumes of a solution containing 15 g per litre of *citric acid* and 8.1 g of *disodium hydrogen phosphate*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 351 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the chromatogram obtained shows three principal peaks and the resolution between each pair of adjacent peaks is at least 3.0.

Inject reference solution (b). The chromatogram obtained shows one principal peak with a signal-to-noise ratio of at least 5.

Inject reference solution (a) and the test solution. Continue the chromatography for four times the retention time of the principal peak in the chromatogram obtained with reference solution (a). In the chromatogram obtained with the test solution, the sum of the areas of any peaks apart from the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (5 per cent). Ignore any peak whose area is less than that of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying (2.4.19). 8 to 12 per cent (aquocobalamin chloride) and 8 to 16 per cent (aquocobalamin sulphate), determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

Assay. *Protect the solutions from light throughout the Assay.*

Weigh a 25 mg and dissolve in sufficient of a solution containing 0.8 per cent v/v of *glacial acetic acid* and 1.09 per cent w/v of *sodium acetate* to produce 1000 ml. Measure the absorbance of the resulting solution at the maximum at about 351 nm (2.4.7). Calculate the content of $C_{62}H_{89}CoN_{13}O_{15}P,HCl$, or of $C_{124}H_{178}Co_2N_{26}O_{30}P_2,H_2SO_4$, taking 190 or 188 respectively, as the specific absorbance at 351 nm.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states whether the contents are aquocobalamin chloride or aquocobalamin sulphate.

Hydroxocobalamin Injection

Hydroxocobalamin Injection is a sterile solution of Hydroxocobalamin in Water for Injections containing sufficient Acetic Acid, Hydrochloric Acid or Sulphuric acid to adjusted to pH 4.

Hydroxocobalamin Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous hydroxocobalamin, $C_{62}H_{89}CoN_{13}O_{15}P$.

Usual strengths. 500 µg per ml; 1 mg per ml.

Identification

Measure the absorbance at about 351 nm and 361 nm; ratio of the absorbance at about 361 nm to that at about 351 nm, about 0.65 (2.4.7).

Tests

pH (2.4.24). 3.8 to 5.5.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and protect them from bright light.

Test solution. Dilute the injection with the mobile phase, if necessary, to obtain a solution having a concentration of 0.05 per cent w/v of hydroxocobalamin.

Reference solution (a). Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 50.0 ml with the mobile phase.

Reference solution (c). Add 0.2 ml of a freshly prepared 2 per cent w/v solution of *chloramine T* and 0.1 ml of *0.05 M hydrochloric acid* to a volume of the injection containing the equivalent of 5 mg of hydroxocobalamin, dilute to 10.0 ml with *water*, shake, allow to stand for 5 minutes and inject immediately.

Chromatographic system

- a stainless steel column 25 cm × 4 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Lichrosorb 100 CH 8/11),
- mobile phase: a mixture of 19.5 volumes of *methanol* and 80.5 volumes of a solution containing 1.5 per cent w/v of *citric acid* and 0.81per cent w/v of *disodium hydrogen orthophosphate*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 351 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the chromatogram obtained shows three principal peaks and the resolution between each pair of adjacent peaks is not less than 3.0.

Inject reference solution (b). The chromatogram obtained shows one principal peak with a signal-to-noise ratio of not less than 5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (10 per cent). Ignore any peak the area of which is less than that of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.4 Endotoxin Unit per µg of hydroxocobalmin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

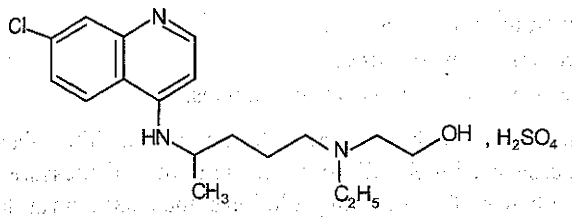
Assay. Dilute a measured volume of the injection containing about 4 mg of anhydrous hydroxocobalamin to 200.0 ml with a

solution containing 0.8 per cent v/v of *glacial acetic acid* and 1.09 per cent w/v of *sodium acetate* and measure the absorbance of the resulting solution at the maximum at about 351 nm (2.4.7). Calculate the content of $C_{62}H_{89}CoN_{13}O_{15}P$ taking 195 as the specific absorbance at 351 nm.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous hydroxocobalamin.

Hydroxychloroquine Sulphate



$C_{18}H_{26}ClN_3O, H_2SO_4$ Mol Wt. 434.0

Hydroxychloroquine Sulphate is 2-[[(RS)-4-[(7-chloroquinolin-4-yl)amino]pentyl](ethyl)amino]ethan-1-ol sulphate.

Hydroxychloroquine Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{26}ClN_3O, H_2SO_4$, calculated on the dried basis.

Category. Antiprotozoal.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum that obtained with *hydroxychloroquine sulphate IPRS* or with the reference spectrum of hydroxychloroquine sulphate.

B. It gives reaction (A) of sulphates (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a). A 0.0001 per cent w/v solution of *hydroxychloroquine sulphate IPRS* in mobile phase A.

Reference solution (b). A solution containing 0.0001 per cent w/v, each of, *desethyl hydroxychloroquine IPRS* and *hydroxychloroquine sulphate IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 90 volumes of *water*, 10 volumes of *acetonitrile* and 0.2 volume of *orthophosphoric acid*,
  B. a mixture of 20 volumes of *water*, 80 volumes of *acetonitrile* and 0.1 volume of *orthophosphoric acid*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
2	100	0
10	85	15
18	0	100
25	0	100
25.1	100	0
50	100	0

Name	Relative retention time	Correction factor
Desethyl hydroxychloroquine[1]	0.92	—
Hydroxychloroquine sulphate	1.0	—
Hydroxychloroquine-O-sulphate[2]	1.4	1.34
4,7- dichloroquinoline	2.8	0.35

[1](*RS*)-2-[[4-[(7-chloro-4-quinolyl)amino] pentyl] amino] ethanol,
[2](*RS*)-2-*N*-[-(7-chloro-4-quinolyl amino) pentyl]–*N*- ethylamino-ethanol-1-(hydrogen sulphate).

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to desethyl hydroxychloroquine and hydroxychloroquine sulphate is not less than 1.0 in the chromatogram obtained with reference solution (b), the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to desethyl hydroxychloroquine is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent), the area of any peak corresponding to hydroxychloroquine-O-sulphate is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any peak corresponding

to 4,7-dichloroquinoline is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent). Ignore the peak due to sulphate and any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides (2.3.12). Dissolve 0.7 g in 15.0 ml of *water*. The solution complies with the limit test for chlorides (350 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Test solution. Dissolve 50 mg of substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution . A 0.01 per cent w/v solution of *hydroxychloroquine sulphate IPRS* in mobile phase A.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{26}ClN_3O, H_2SO_4$.

Storage. Store protected from light.

Hydroxychloroquine Tablets

Hydroxychloroquine Sulphate Tablets

Hydroxychloroquine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hydroxychloroquine sulphate, $C_{18}H_{26}ClN_3O, H_2SO_4$.

Usual strength. 200 mg

Identification

A. Dissolve a quantity of the powdered tablets containing 0.1 g of Hydroxychloroquine Sulphate in a mixture of 10 ml of *water* and 2 ml of *2M sodium hydroxide* and extract with two 20-ml quantities of *chloroform*. Wash the Combined chloroform extract with *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The resulting solution complies with the following test

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydroxychloroquine sulphate IPRS* treated in the same manner or with the reference spectrum of hydroxychloroquine.

B. Shake a quantity of the powdered tablets containing 0.1 g of Hydroxychloroquine Sulphate with 10 ml of *water* and filter. To the filterate add 1 ml of *2M hydrochloric acid* and 1 ml of *barium chloride solution*. A white precipitate is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, dilute if necessary with the dissolution medium and measure the absorbance of the resulting solution at the maximum at about 343 nm (2.4.7). Calculate the content of $C_{18}H_{26}ClN_3O, H_2SO_4$ in the medium from the absorbance obtained from a solution of known concentration of *hdroxychloroquine sulphate IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{18}H_{26}ClN_3O, H_2SO_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 200 mg of Hydroxychloroquine Sulphate in 150 ml of mobile phase A, dilute to 200.0 ml with mobile phase A and filter. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with mobile phase A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with mobile phase A.

Reference solution (c). A 0.00005 per cent w/v solution of *2-[4-[(7-chloro-4-quinolinyl)amino]pentyl]aminoethanol IPRS* in mobile phase A.

Reference solution (d). A solution containing 0.0001 per cent, w/v each, of *hydroxychloroquine sulphate IPRS* and *2-[4-[(7-chloro- 4-quinolinyl)amino]pentyl]aminoethanol IPRS* in mobile phase A.

Use the chromatographic system as described under Assay.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to 2-[4-[(7-chloro-4-quinolinyl)amino]pentyl]aminoethanol and hydroxychloroquine is not less than 1.5.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-[4-[(7-chloro-4- quinolinyl)amino] pentyl]aminoethanol is not more than the area of principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The sum of the areas of other secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 200 mg of Hydroxychloroquine Sulphate with 150 ml of mobile phase A, dilute to 200.0 ml and filter. Dilute 1.0 ml of the filtrate to 10.0 ml with mobile phase A.

Reference solution (a). A 0.01 per cent w/v solution of *hydroxychloroquine sulphate IPRS* in mobile phase A.

Reference solution (b). A 0.0001 per cent w/v solution, each of, *hydroxychloroquine sulphate IPRS* and *2-[4-[(7-chloro-4-quinolinyl)amino]pentyl]aminoethanol IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: A. a mixture of 90 volumes of *water*, 10 volumes of *acetonitrile*, and 0.2 volume of *orthophosphoric acid*,
  B. a mixture of 20 volumes of *water*, 80 volumes of *acetonitrile*, and 0.1 volume of *orthophosphoric acid*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

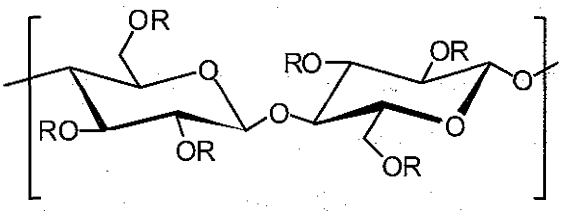
Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
2	100	0
10	85	15
18	100	0
25	100	0

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to 2-[4-[(7-chloro-4-quinolinyl)amino]pentyl]aminoethanol and hydroxychloroquine is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{26}ClN_3O, H_2SO_4$.

Hydroxyethylcellulose



R = -H or $—CH_2CH_2OH$

Hydroxyethylcellulose is cellulose, 2-hydroxyethyl ether.

Hydroxyethylcellulose is a partially substituted poly(hydroxyethyl) ether of cellulose. It is available in several grades, varying in viscosity and degree of substitution, and some grades are modified to improve their dispersion in water. It may contain suitable anticaking agents.

Category. Pharmaceutical aid.

Description. A white to light tan; hygroscopic powder or granules.

Identification

A. Stir 1 g into 100 ml of *water*, it is dissolved completely to produce a colloidal solution that remains clear when heated to 60°.

B. Place 1 ml of the solution from test A on a glass plate and allow the water to evaporate; a thin, self-sustaining film is formed.

C. To 1 ml of 0.05 per cent w/v solution, add 1 ml of 5.0 per cent w/v *phenol* solution. Add 5 ml of *sulphuric acid*, shake and allow to cool; the colour of the solution so obtained becomes orange.

Tests

pH (2.4.24). 6.0 to 8.5, determined in a 1.0 per cent w/v solution.

Viscosity (2.4.28). 50.0 per cent to 150.0 per cent of the labelled viscosity.

Lead (2.3.15). Not more than 0.001 per cent.

Heavy metals (2.3.13). 1 g complies with the test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 5.0 per cent.

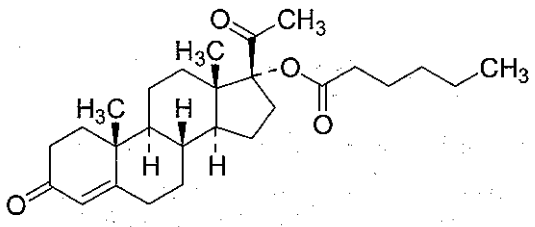
Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Storage. Store protected from moisture.

Labelling. The label indicates its viscosity, under specified conditions, in aqueous solution. The indicated viscosity may be in the form of a range encompassing 50 per cent to 150 per cent of the average value.

Hydroxyprogesterone Hexanoate

Hydroxyprogesterone Caproate



$C_{27}H_{40}O_4$ Mol. Wt. 428.6

Hydroxyprogesterone Hexanoate is 3,20-dioxopregn-4-en-17α-yl hexanoate

Hydroxyprogesterone Hexanoate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{27}H_{40}O_4$, calculated on the dried basis.

Category. Progestogen.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydroxyprogesterone hexanoate IPRS* or with the reference spectrum of hydroxyprogesterone hexanoate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *1,2-propane diol*.

Mobile phase. A mixture of equal volumes of *cyclohexane* and *light petroleum (40° to 60°)*.

Test solution. Dissolve 25 mg of the substance under examination in 10.0 ml of the same solvent mixture.

Reference solution (a). Dissolve 25 mg of *hydroxyprogesterone hexanoate IPRS* in 10.0 ml of the same solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Dissolve 1 mg in 1 ml of *sulphuric acid* and allow to stand for 2 minutes; a faint yellow colour is produced which, on the addition of 0.5 ml of *water*, changes first to green, then to red and finally to reddish-violet with a blue fluorescence.

D. Heat 50 mg with 2 ml of *0.5 M ethanolic potassium hydroxide* in a water-bath for 5 minutes. Add 3 ml of *water*, evaporate the ethanol, add 2 ml of *sulphuric acid (50 per cent)* and heat on a water-bath; the odour of hexanoic acid is produced.

Tests

Acidity. Dissolve 0.2 g in 25 ml of *ethanol* previously neutralised to *bromothymol blue solution* and titrate immediately with *0.01 M sodium hydroxide* until a faint blue colour is produced. Not more than 1.0 ml of *0.01 M sodium hydroxide* is required.

Specific optical rotation (2.4.22). +44.0° to +49.0°, determined in a 2.0 per cent w/v solution in *dioxan*.

Related foreign steroids. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of equal volumes of *cyclohexane* and *ethyl acetate*.

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *chloroform*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained

with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.1 per cent, determined on 2.0 g

Assay. Weigh 60 mg and dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 5.0 ml to 250.0 ml with *ethanol* and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{27}H_{40}O_4$ taking 395 as the specific absorbance at 240 nm.

Storage. Store protected from light.

Hydroxyprogesterone Injection

Hydroxyprogesterone Caproate Injection;
Hydroxyprogesterone Hexanoate Injection

Hydroxyprogesterone Injection is a sterile solution of Hydroxyprogesterone Hexanoate in a suitable ester, in a suitable fixed oil or in any mixture of fixed oils.

Hydroxyprogesterone Hexanoate Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hydroxyprogesterone hexanoate, $C_{27}H_{40}O_4$.

Usual strength. 250 mg per ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of equal volumes of *cyclohexane* and *ethyl acetate*.

Test solution. Dilute the injection with *chloroform* to give a solution containing 1.0 per cent w/v of Hydroxyprogesterone Hexanoate.

Reference solution. A solution containing 1 per cent w/v of *hydroxyprogesterone hexanoate IPRS* in *chloroform*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Ignore any spots due to the vehicle.

B. Dissolve a volume of the injection containing 0.1 g of Hydroxyprogesterone Hexanoate in 10 ml of *light petroleum (40° to 60°)* and extract with three quantities, each of 10 ml, of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *light petroleum (40° to 60°)*, dilute with *water* until the solution becomes turbid and allow to stand in ice for about 2 hours until a white precipitate is produced. The precipitate, after washing with *water*, melts at about 120° (2.4.21).

Tests

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a quantity of the injection containing about 0.125 g of Hydroxyprogesterone Hexanoate add sufficient *chloroform* to produce 100.0 ml. Dilute 5.0 ml to 100.0 ml with *chloroform*; to 5.0 ml add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20.0 ml. Allow to stand for 45 minutes and measure the absorbance of the resulting solution at the maximum at about 380 nm (2.4.7), using as the blank 5 ml of *chloroform* treated in the same manner. Calculate the content of $C_{27}H_{40}O_4$ from the absorbance obtained by repeating the operation using a 0.00625 per cent w/v solution of *hydroxyprogesterone hexanoate IPRS* in *chloroform* and beginning at the words "to 5.0 ml add..."

Storage. Store protected from light.

Labelling. The label states that the preparation is intended for intramuscular injection only.

Hydroxypropyl Cellulose

Cellulose, 2-Hydroxypropyl Ether; Hyprolose

Hydroxypropylcellulose is a cellulose having some of the hydroxyl groups in the form of the 2-hydroxypropyl ether. It may contain not more than 0.6 per cent of silica (SiO_2). The various grades commercially available are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/w solution measured at 20°.

Category. Pharmaceutical aid (tablet excipient; suspending agent).

Description. A white or yellowish white powder; hygroscopic after drying.

Identification

A. With constant stirring add a quantity equivalent to 1 g of the dried substance into 50 ml of *carbon dioxide-free water* previously heated to 90°. Allow to cool, dilute to 100.0 ml with *carbon dioxide-free water* and continue stirring until solution is complete (solution A). Heat 10 ml of solution A on a water-bath with stirring. At temperatures above 40° the solution becomes cloudy or a flocculent precipitate is formed. On cooling, the solution becomes clear.

B. To 10 ml of solution A add 0.3 ml of *2 M acetic acid* and 2.5 ml of a 10 per cent w/v solution of *tannic acid*; a yellowish white, flocculent precipitate is produced which dissolves in *6 M ammonia*.

C. Without heating completely dissolve 0.2 g in 15 ml of a 70 per cent w/w solution of *sulphuric acid*, pour the solution with stirring into 100 ml of iced *water*. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of *sulphuric acid*, added dropwise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of *ninhydrin* in 100 ml of a 4.55 per cent w/v solution of *sodium metabisulphite*, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

D. Place 1 ml of solution A on a glass plate. After evaporation of the *water* a thin film is produced.

Tests

Appearance of solution. Solution A is not more opalescent than opalescence standard OS3 (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 5.0 to 8.5, determined in solution A.

Apparent viscosity. 75 to 140 per cent of the stated value, determined by the following method. Weigh a quantity equivalent to 2.0 g of the dried substance and add, with constant stirring, to 50 ml of *water* previously heated to 90°. Allow to cool, dilute to 100.0 ml with *water* and continue stirring until solution is complete. Adjust the weight of the solution to 100 g and centrifuge the solution to expel any trapped air. Determine the viscosity, Method C, at 20° using a shear rate of 10 s^{-1} (2.4.28). For a product of low viscosity, use a quantity of the substance under examination sufficient to prepare a solution of the concentration stated on the label.

Chlorides (2.3.12). Dilute 5.0 ml of solution A to 15 ml with *water*. The resulting solution complies with the limit test for chlorides (0.5 per cent).

Silica. Not more than 0.6 per cent, determined by the following method. To the residue obtained in the test for Sulphated ash add sufficient *ethanol (95 per cent)* to moisten the residue completely. Add 6 ml of *hydrofluoric acid* in small portions. Evaporate to dryness at 95° to 105° taking care to avoid loss by spurting. Cool and rinse the walls of the platinum crucible with 6 ml of *hydrofluoric acid*. Add 0.5 ml of *sulphuric acid* and evaporate to dryness. Progressively increase the temperature, ignite at 900°, allow to cool in a desicator and weigh. The difference between the weight of the residue obtained in the test for Sulphated ash and the weight of the final residue is equal to the amount of silica in the substance under examination.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 1.6 per cent, determined on 1.0 g in a platinum crucible.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Storage. Store protected from moisture.

Labelling. The label states the apparent viscosity in millipascal seconds of a 2 per cent w/w solution. For products of low viscosity the label also states the concentration of the solution to be used and the apparent viscosity in millipascal seconds.

Hydroxypropylmethylcellulose

Cellulose, 2-Hydroxypropylmethyl Ether; Hypromellose

Hydroxypropylmethylcellulose is a cellulose having some of the hydroxyl groups in the form of the methyl ether and some in the form of the 2-hydroxypropyl ether. The various grades commercially available are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/v solution measured at 20°.

Category. Treatment of tear deficiency; pharmaceutical aid (tablet excipient; suspending agent).

Description. A white or yellowish white, fibrous or granular powder; hygroscopic after drying.

Identification

A. With constant stirring add a quantity containing 1 g of the dried substance into 50 ml of *carbon dioxide-free water* previously heated to 90°. Allow to cool, dilute to 100 ml with *carbon dioxide-free water* and continue stirring until solution is complete (solution A). Heat 10 ml of solution A in a water-bath with stirring. At temperatures above 50° the solution becomes cloudy or a flocculent precipitate is formed. On cooling, the solution becomes clear or slightly opalescent.

B. To 10 ml of solution A add 10 ml of *1 M sodium hydroxide* or *1 M hydrochloric acid*; in either case the mixture remains stable.

C. To 10 ml of solution A add 0.3 ml of *2 M acetic acid* and 2.5 ml of a 10 per cent w/v solution of *tannic acid*; a yellowish white, flocculent precipitate is produced which dissolves in *6 M ammonia*.

D. Without heating completely dissolve 0.2 g in 15 ml of a 70 per cent w/w solution of *sulphuric acid*, pour the solution with stirring into 100 ml of iced *water*. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of *sulphuric acid*, added dropwise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of *ninhydrin* in 100 ml of a 4.55 per cent w/v solution of *sodium metabisulphite*, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

E. Place 1 ml of solution A on a glass plate. After evaporation of the *water* a thin film is produced.

Tests

pH (2.4.24). 5.5 to 8.0, determined in solution A.

Appearance of solution. Solution A is not more opalescent than opalescence standard OS3 (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Apparent viscosity. 75 to 140 per cent of the stated value, determined by the following method. Weigh a quantity equivalent to 2.0 g of the dried substance and add, with constant stirring, to 50 ml of *water* previously heated to 90°. Allow to cool, dilute to 100.0 ml with *water* and continue stirring until solution is complete. Adjust the weight of the solution to 100 g and centrifuge the solution to expel any trapped air. Determine the viscosity, Method C, at 20° using a shear rate of 10 s^{-1} (2.4.28).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides (2.3.12). Dilute 5.0 ml of solution A to 15 ml with *water*. The resulting solution complies with the limit test for chlorides (0.5 per cent).

Sulphated ash (2.3.18). Not more than 3.0 per cent.

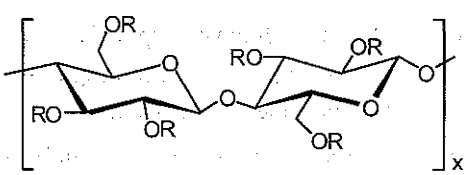
Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 0.5 g by drying in an oven at 105°.

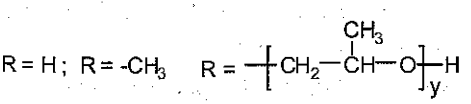
Storage. Store protected from moisture.

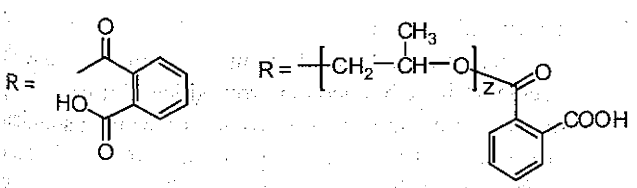
Labelling. The label states the apparent viscosity in millipascal seconds of a 2 per cent w/v solution.

Hydroxypropyl Methylcellulose Phthalate

Hypromellose Phthalate







Hydroxypropyl Methylcellulose Phthalate is 2-hydroxypropyl methylcellulose phthalate.

Category. Pharmaceutical aid.

Description. A white to off-white, free flowing flakes or a granular powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydroxypropyl methylcellulose phthalate IPRS* or with the reference spectrum of hydroxypropyl methylcellulose phthalate.

Tests

Viscosity (2.4.28). Not less than 80 per cent and not more than 120 per cent of the label claim at 20° by dissolving 10 g, previously dried at 105° for 1 hour, in 90 g of a mixture of equal weights of *methanol* and *dichloromethane*.

Free phthalic acid. Not more than 1.0 per cent.

Test solution. Disperse 200 mg of the substance under examination with 50 ml of *acetonitrile*, with the aid of ultrasound. Add 10 ml of *water*, mix and dilute to 100.0 ml with *acetonitrile*.

Reference solution. Disperse 12.5 mg of *phthalic acid* with 125 ml of *acetonitrile*. Add 25 ml of *water* and dilute to 250.0 ml with *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of *0.1 M cyanoacetic acid* and 15 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of phthalic acid.

Phthalyl content. Not less than 21.0 per cent and not more than 35.0 per cent.

Transfer 1 g to a conical flask, dissolve in 50 ml of a mixture of 2 volume of *ethanol*, 2 volume of *acetone* and 1 volume of *water*. Add *phenolphthalein TS* and titrate with *0.1 M sodium hydroxide*. Carry out a blank titration.

Calculate the content of phthalyl by using formula:

$$0.01(149.1)(V/W) - 2(149.1/166.1)(P)$$

where 149.1 and 166.1 are the molecular weights of the phthalyl group and phthalic acid respectively,

V is the volume, in ml of *0.1 M sodium hydroxide* consumed after correction for the blank,

W is the weight, in g, calculated on the anhydrous basis of Hypromellose Phthalate taken,

and P is the percentage of free phthalic acid found as directed in the test for Limit of free phthalic acid.

Chlorides. Dissolve 2.0 g in 40 ml of *0.2 M sodium hydroxide*, add 1 drop of *phenolphthalein TS*, and add *2 M nitric acid* dropwise, with stirring, until the red colour is discharged. Add an additional 20 ml of *2 M nitric acid* with stirring. Heat on a water-bath, with stirring, until the gel-like precipitate formed becomes granular. Cool the mixture, and centrifuge. Separate the liquid phase, and wash the residue with three successive 20-ml portions of *water*, separating the washings by centrifuging. Dilute the combined liquids with *water* to 200 ml, mix, and filter. 35 ml of the filtrate complies with the limit test for chlorides (0.07 per cent).

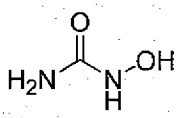
Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Storage. Store protected from moisture.

Hydroxyurea



$CH_4N_2O_2$ Mol. Wt. 76.1

Hydroxyurea contains not less than 97.0 per cent and not more than 103.0 per cent of $CH_4N_2O_2$, calculated on the dried basis.

Category. Anticancer.

Description. A white to off-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydroxyurea IPRS* or with the reference spectrum of hydroxyurea.

Tests

Urea and related compounds. Determine by paper chromatography (2.4.15).

Mobile phase. A mixture of equal volumes of *isobutyl alcohol* and *water*, mix, use upper layers as the mobile phase and lower layers as stationary phase.

Test solution. Dissolve 10.0 mg of the substance under examination in 1.0 ml of *water*.

Reference solution. A 0.01 per cent w/v solution of *urea IPRS* in *water*.

Use a suitable chromatographic paper strip by dipping it in pH 6.5 buffer solution (prepared by mixing of 700 ml of *0.2 M dibasic sodium phosphate* and 300 ml of *0.1 M citric acid*). Dry the paper strip, and apply 100 µl of the test solution and 50 µl of the reference solution. Place the strip in a chromatographic chamber for descending chromatography containing the stationary phase in the bottom of the chamber and the mobile phase in the trough. Develop for 24 hours, remove the strip from the chamber, dry in air again develop from 24 hours. Remove the strip, air dry, spray with *p-dimethylaminobenzaldehyde* solution 1.0 per cent (Dissolving 1.0 g of *p-dimethylaminobenzaldehyde* in 50 ml of *alcohol*, add 2 ml of *hydrochloric acid* and dilute with *alcohol* to 100.0 ml), heat at 90° for 1 to 2 minutes. Not more than two spots, other than the major component, are present in the test solution and their intensities are not greater than the intensity of the spot from the reference solution (0.5 per cent of each impurity). The Rf values relative to *hydroxyurea*, the principal spot, are 0.65 and 1.26 (*urea*).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in vacuum at 60° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 40 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A solution containing 0.04 per cent w/v each of of *hydroxyurea IPRS* and *hydroxylamine hydrochloride* in the mobile phase.

Reference solution (b). A 0.04 per cent w/v solution of *hydroxyurea IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column of 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.7 g of *tetrabutylammonium hydrogen sulphate* and 1.74 g of *dibasic potassium*

phosphate anhydrous in 1000 ml of *water*, adjusted to pH 5.0 with *1M sodium hydroxide or* dilute *orthophosphoric acid* and 15 volumes of *methanol*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to hydroxylamine and hydroxyurea is not less than 1.5.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 5000, tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate content of $CH_4N_2O_2$.

Storage. Store protected from moisture.

Hydroxyurea Capsules

Hydroxyurea Capsules contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hydroxyurea, $CH_4N_2O_2$.

Usual strength. 500 mg.

Identification

Shake a quantity of the contents of the capsules containing 30 mg of Hydroxyurea, to a centrifuge tube, add 10 ml of *anhydrous methanol*. Mix and centrifuge for 3 minutes. Transfer 1.0 ml of the clear supernatant to a mortar containing 500 mg of *potassium bromide*, triturate to a homogeneous blend, dry in a vacuum desiccators at 60° for 3 hours, and prepare a suitable disk.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydroxyurea IPRS* treated in the same manner or with the reference spectrum of hydroxyurea.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of medium and filter.

Determine by liquid chromatography (2.4.14) using the chromatographic conditions as described under Assay.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium. Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate.

Reference solution. Prepare a solution using *hydroxyurea IPRS* in the dissolution medium to obtain a solution having concentration similar to the expected concentration in the test solution.

Inject the reference solution and the test solution.

Calculate the content of $CH_4N_2O_2$.

Q. Not less than 80 per cent of the stated amounts of $CH_4N_2O_2$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing 40 mg of Hydroxyurea and transfer to a 100.0 ml volumetric flask. Add about 75 ml of mobile phase and disperse with the aid of ultrasound for about 10 minutes. Stir the solution with magnetic stirrer for further 30 minutes, cool and dilute to volume, filter, rejecting the first few ml of filtrate.

Reference solution (a). A solution containing 0.04 per cent w/v each of *hydroxyurea IPRS* and *hydroxylamine hydrochloride* in the mobile phase.

Reference solution (b). A 0.04 per cent w/v solution of *hydroxyurea IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column of 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.7 g of *tetrabutylammonium hydrogen sulphate* and 1.74 g of *dibasic potassium phosphate anhydrous* in 1000 ml of *water*, adjusted to pH 5.0 with *dilute sodium hydroxide or* dilute *orthophosphoric acid* and 15 volumes of *methanol*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 10 µl.

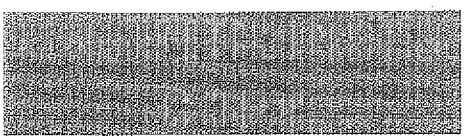
Inject reference solution (a). The test is not valid unless the resolution between the peaks due to hydroxylamine and hydroxyurea is not less than 1.5.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 5000, tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

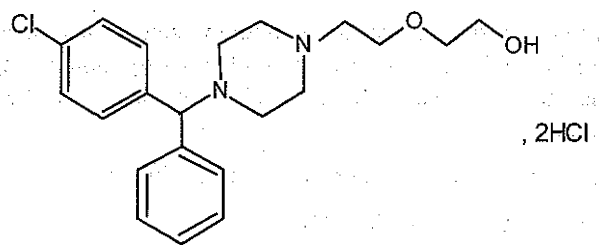
Inject reference solution (b) and the test solution.

Calculate content of $CH_4N_2O_2$ in the capsules.

Storage. Store protected from moisture.



Hydroxyzine Hydrochloride



$C_{21}H_{27}ClN_2O_2,2HCl$ Mol. Wt. 447.8

Hydroxyzine Hydrochloride is (*RS*)-2-[2-[4-[(4-chlorophenyl) phenylmethyl]piperazin-1-yl]ethoxy]ethanol dihydrochloride.

Hydroxyzine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{27}ClN_2O_2,2HCl$, calculated on the dried basis.

Category. Antiallergic.

Description. A white or almost white, hygroscopic, crystalline powder.

Identification

Tests B and C may be omitted if tests A and D are carried out. Test A may be omitted if tests B, C and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hydroxyzine hydrochloride IPRS* or with the reference spectrum of hydroxyzine hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 1 volume of *ammonia*, 24 volumes of *ethanol (95 per cent)* and 75 volumes of *toluene*.

Solvent mixture. 50 volumes of *methanol* and 50 volumes of *dichloromethane*.

Test solution. Dissolve 0.5 g of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). A 5.0 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in the solvent mixture.

Reference solution (b). A 5.0 per cent w/v solution of *meclozine dihydrochloride* in the solvent mixture. Dilute 1.0 ml of the solution to 2.0 ml with reference solution (a).

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with *potassium iodobismuthate solution* and examine immediately. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated principal spots.

C. Dissolve 0.1 g in *ethanol (95 per cent)* and dilute to 15 ml with the same solvent. Add 15 ml of a saturated solution of *picric acid* in *ethanol (95 per cent)*. Allow to stand for 15 minutes; a precipitate is formed, filter. Recrystallise from *ethanol (95 per cent)*. Initiate crystallization, if necessary, by scratching the wall of the tube with a glass rod. The crystals melt (2.4.21) at 189° to 192°.

D. It gives reaction (A) of chlorides (2.3.1).

Tests

Solution A. A 10.0 per cent w/v solution in *water*.

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

Optical rotation (2.4.22). - 0.1° to + 0.1°, determined on solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in the mobile phase.

Reference solution (b). Dilute 3.0 ml of the test solution to 200.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 25.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with base-deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: dissolve 0.5 g of *sodium methanesulphonate* in a mixture of 14 volumes of *triethylamine*, 300 volumes of *acetonitrile* and 686 volumes of *water*, adjusted to pH 2.7 with *sulphuric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the peak-to-valley ratio is not less than 10.0, where H_p is the height above the baseline of the peak immediately before the peak due to hydroxyzine and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to hydroxyzine.

Inject reference solution (b) and the test solution. Run the chromatogram 2.5 times the retention time of the principal

peak. The area of any secondary peak is not more than 0.33 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method A (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.2 g in 10 ml of *anhydrous acetic acid* and 40 ml of *acetic anhydride*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02239 g of $C_{21}H_{29}Cl_3N_2O_2$.

Storage. Store protected from light and moisture.

Hydroxyzine Oral Solution

Hydroxyzine Oral Solution contains Hydroxyzine Hydrochloride in a suitable vehicle.

Hydroxyzine Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of hydroxyzine hydrochloride,, $C_{21}H_{27}ClN_2O_2,2HCl$.

Usual strength. 10 mg per 5 ml.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 1 volume of *dichloromethane* and 1 volume of *methanol*.

Mobile phase. A mixture of 1 volume of *13.5 M ammonia*, 24 volumes of *ethanol* and 75 volumes of *toluene*.

Test solution. Disperse a quantity of the oral solution containing 50 mg of Hydroxyzine Hydrochloride with 5 ml of solvent mixture and dilute to 50.0 ml with the solvent mixture, centrifuge and use the supernatant liquid.

Reference solution (a). A 1.0 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.5 per cent w/v, each of, *hydroxyzine hydrochloride IPRS* and *meclozine hydrochloride IPRS* in the solvent mixture.

Apply to the plate 30 µl of the test solution and 2 µl of reference solution (a) and (b). Allow the mobile phase to rise 15 cm. Dry the plate in air, spray with *potassium iodobismuthate solution*, heat at 110° for 5 minutes and allow to cool. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Tests

pH (2.4.24). 2.7 to 3.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the oral solution containing 50 mg of Hydroxyzine Hydrochloride with 10 ml of mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in the mobile phase.

Reference solution (c). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 14 volumes of *triethylamine*, 300 volumes of *acetonitrile* and 686 volumes of a 0.075 per cent w/v solution of *sodium methanesulphonate*, adjusted to pH 2.7 with *sulphuric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

The retention time of hydroxyzine is about 9 minutes.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 10, where H_p is the height above the baseline of the peak immediately before the peak due to hydroxyzine and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to hydroxyzine.

Inject reference solution (a), (c) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal

peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the oral solution containing 50 mg of Hydroxyzine Hydrochloride with 10 ml of *water* for 20 minutes, add 125 ml of *methanol* and shake for a further 30 minutes and dilute to 250.0 ml with 30 per cent v/v solution of *acetonitrile*, filter. Dilute 5.0 ml of the solution to 10.0 ml with a 30 per cent v/v solution of *acetonitrile*.

Reference solution (a). A 0.01 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in 30 per cent v/v solution of *acetonitrile*.

Reference solution (b). A 0.01 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 10, where H_p is the height above the baseline of the peak immediately before the peak due to hydroxyzine and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to Hydroxyzine.

Inject reference solution (a) and the test solution.

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_{21}H_{27}ClN_2O_2,2HCl$.

Hydroxyzine Tablets

Hydroxyzine Hydrochloride Tablets

Hydroxyzine Tablets contain Hydroxyzine Hydrochloride.

Hydroxyzine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of hydroxyzine hydrochloride, $C_{21}H_{27}ClN_2O_2,2HCl$.

Usual strengths. 10 mg; 25 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 1 volume of *13.5 M ammonia*, 24 volumes of *ethanol* and 75 volumes of *toluene*.

Solution A. 1 volume of *methanol* and 1 volume of *dichloromethane*.

Test solution. Shake a quantity of the powdered tablets containing 100 mg of Hydroxyzine Hydrochloride with 5 ml of solution A and dilute to 10 ml with solution A, centrifuge and use the supernatant liquid.

Reference solution (a). A 1.0 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in solution A.

Reference solution (b). A solution containing 0.5 per cent w/v, each of, *hydroxyzine hydrochloride IPRS* and *meclozine hydrochloride IPRS* in solution A.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air spray with *potassium iodobismuthate solution*, heat at 110° for 5 minutes and allow to cool. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary, with the medium. Measure the absorbance of the resulting solution at the maximum at about 230 nm (2.4.7). Calculate the content of $C_{21}H_{27}ClN_2O_2,2HCl$ in the medium from the absorbance obtained from a solution of known concentration of *hydroxyzine hydrochloride IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{27}ClN_2O_2,2HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Hydroxyzine Hydrochloride with 10 ml of the mobile phase and dilute to 50.0 ml with the mobile phase, filter.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in the mobile phase.

Reference solution (c). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 14 volumes of *triethylamine*, 300 volumes of *acetonitrile* and 686 volumes of a 0.075 per cent w/v solution of *sodium methanesulphonate*, adjusted to pH 2.7 with *sulphuric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (a), (c) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Hydroxyzine Hydrochloride with 10 ml of *water* for 20 minutes, add 125 ml of *methanol* and shake for a further 30 minutes and dilute to 250.0 ml with 30 per cent v/v solution of *acetonitrile*, filter. Dilute 5.0 ml of the filtrate to 10.0 ml with 30 per cent v/v solution of *acetonitrile*.

Reference solution (a). A 0.01 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in a 30 per cent v/v solution of *acetonitrile*.

Reference solution (b). A 0.01 per cent w/v solution of *hydroxyzine hydrochloride IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

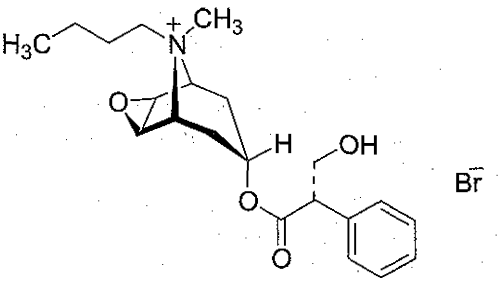
Inject reference solution (b). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing fcator is not more than 2.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{21}H_{27}ClN_2O_2,2HCl$ in the tablets.

Hyoscine Butylbromide

Scopolamine Butylbromide



$C_{21}H_{30}BrNO_4$ Mol. Wt. 440.4

Hyoscine Butylbromide is (1*S*,3*s*,5*R*,6*R*,7*S*,8*r*)-6,7-epoxy-8-butyl-3-[(*S*)-tropoyloxy]tropanium bromide.

Hyoscine Butylbromide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{21}H_{30}BrNO_4$, calculated on the dried basis.

Category. Parasympatholytic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hyoscine butylbromide IPRS* or with the reference spectrum of hyoscine butylbromide.

B. It gives the reactions of bromides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 5.5 to 6.5, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). −20.0° to −18.0°, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in mobile phase B and dilute to 10.0 ml with mobile phase B.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase B. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase B.

Reference solution (b). Dissolve 5 mg *of hyoscine butylbromide for system suitability IPRS* (containing impurity A and B) in mobile phase B and dilute to 10.0 ml with mobile phase B.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with silica gel bonded to alkyl groups (1.8 µm),
- column temperature: 50°,
- mobile phase: A. a mixture of 5 volumes of *acetonitrile* and 95 volumes of a 0.2 per cent v/v solution of *perchloric acid*,

  B. a mixture of 30 volumes of a 0.2 per cent v/v solution of *perchloric acid* and 70 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 2 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	91	9
1	91	9
4.2	75	25
5.5	66	34
10	15	85
11	15	85
12	91	9
15	91	9

Name	Relative retention time
Bromide	0.1
Hyoscine impurity B[1]	0.28
Hyoscine impurity A[2]	0.37
Hyoscine butylbromide (Retention time: about 6 minutes)	1.0

[1](2*RS*)-3-hydroxy-2-phenylpropanoic acid (DL-tropic acid),

[2](*R*,2*R*,4*S*,5*S*,7*S*)-9-methyl-3-oxa-9-azatricyclo[3.3.1.02,4] nonan-7-yl (2*S*)-3-hyroxy-2-phenylpropanoate (hyoscine).

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to hyoscine impurity B and hyoscine impurity A is not less than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to hyoscine impurity A is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of the areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent). Ignore the peak due to bromide and any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 2.5 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Dissolve 0.6 g in 30 ml of *anhydrous glacial acetic acid* and add 5 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04404 g of $C_{21}H_{30}BrNO_4$.

Storage. Store at a temperature not exceeding 30° protected from light and moisture.

Hyoscine Butylbromide Injection

Scopolamine Butylbromide Injection

Hyoscine Butylbromide Injection is a sterile solution of Hyoscine Butylbromide in Water for Injections.

Hyoscine Butylbromide Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hyoscine butylbromide, $C_{21}H_{30}BrNO_4$.

Usual strength. 20 mg per ml.

Identification

Evaporate to dryness a volume of the injection containing 0.1 g of Hyoscine Butylbromide, shake the residue with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness and triturate the residue with 5 ml of *acetonitrile*. Evaporate to dryness and dry the residue at 50° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hyoscine butylbromide IPRS* or with the reference spectrum of hyoscine butylbromide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.15 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima at about 252 nm, 257 nm and 264 nm and a less well-defined maximum at about 247 nm.

C. To 1 mg add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

Tests

pH (2.4.24). 3.7 to 5.5.

Hyoscine. Determine by liquid chromatography (2.4.14).

Test solution. The injection diluted if necessary, to contain 1.0 per cent w/v of Hyoscine Butylbromide in *0.001 M hydrochloric acid.*

Reference solution (a). A 0.001 per cent w/v solution of *hyoscine hydrobromide IPRS* in *0.001 M hydrochloric acid.*

Reference solution (b). Add 10 µl of the test solution to 10 ml of reference solution (a).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (10 µm),
- mobile phase: dissolve 2.0 g of *sodium dodecyl sulphate* in a mixture of 370 ml of *0.001M hydrochloric acid* and 680 ml of *methanol,*
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to hyoscine and butylhyoscine is not less than 5.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to hyoscine is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing 100 mg of Hysoscine Butylbromide to 20.0 ml with *0.001 M hydrochloric acid.*

Reference solution (a). A 0.005 per cent w/v solution of *hysoscine butylbromide IPRS* in *0.001 M hydrochloric acid.*

Reference solution (b). A 0.006 per cent w/v solution of *tropic acid* in *0.001 M hydrochloric acid.*

Reference solution (c). A solution containing 0.5 per cent w/v of *hysoscine butylbromide IPRS* and 0.003 per cent w/v of *tropic acid* in *0.001 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 12.5 cm × 4 mm, packed with octadecylsilane bonded to porous silica (5 µm), (Such as LiChrospher 60 RP- Select B),
- column temperature: 45°,
- mobile phase: a mixture of 87.5 volumes of a buffer solution prepared by dissolving 12.65 g of *sodium dihydrogen orthophosphate monohydrate* and 3.4 g of *tetrabutylammonium hydrogen sulphate*, in 1000 ml of *water*, adjusted to pH 5.5 with *0.5 M sodium hydroxide* and 12.5 volumes of *methanol,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Hyoscine butylbromide (Retention time: about 5 minutes)	1.0	—
Tropic acid	1.3	—
Hyoscine impurity G[1]	2.6	0.41

[1](1*R*,2*R*,4*S*,5*S*,7s,9r)-9-butyl-9-methyl-7-[(2-phenylprop-2-enoyl)oxy]-3-oxa-9-azoniatricyclo[3.3.1$0^{2,4}$]nonane (apo-N-butylhyoscine).

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to hyoscine butylbromide and tropic acid is not less than 1.5.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to hyoscine impurity G is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of any peak corresponding to tropic acid is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.2 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent). Ignore the peak due to bromide and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Bacterial endotoxins (2.2.3). Not more than 8.75 Endotoxin Units per mg of hyoscine butylbromide.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing about 40 mg of Hyoscine Butylbromide in 100.0 ml of *0.001M hydrochloric acid.*

Reference solution. A 0.04 per cent w/v solution of *hyoscine butylbromide IPRS* in *0.001M hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (10 µm) (Such as Lichrosorb C8),
- mobile phase: a buffer solution prepared by dissolving 2.0 g of *sodium lauryl sulphate* in a mixture of 370 volumes of *0.001 M hydrochloric acid* and 680 volumes of *methanol,*

- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0 per cent and the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{30}BrNO_4$ in the injection.

Storage. Store at a temperature not exceeding 30° protected from light and moisture.

Hyoscine Butylbromide Tablets

Scopolamine Butylbromide Tablets

Hyoscine Butylbromide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hyoscine butylbromide, $C_{21}H_{30}BrNO_4$.

Usual strength. 10 mg.

Identification

Shake a quantity of the powdered tablets containing 50 mg of Hyoscine Butylbromide with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness and triturate the residue with 5 ml of *acetonitrile*. Evaporate to dryness and dry the residue at 50° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hyoscine butylbromide IPRS* or with the reference spectrum of hyoscine butylbromide.

B. To 1 mg add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.001M hydrochloric acid*,
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate with the dissolution medium to obtain a solution containing 0.002 per cent w/v of Hyoscine Butylbromide.

Reference solution. A 0.002 per cent w/v solution of *hyoscine butylbromide IPRS* in the dissolution medium.

Chromatographic system
- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm), (Such as Lichrospher 60 RP select B),
- mobile phase: a mixture of 78 volumes of a buffer solution prepared by dissolving 2.5 g of *sodium heptanesulphonate monohydrate* in 720 ml of a 0.908 per cent w/v solution of *potassium dihydrogen orthophosphate* and add 60 ml of 1.188 per cent w/v solution of *disodium hydrogen orthophosphate* and 24 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{30}BrNO_4$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{30}BrNO_4$.

Hyoscine. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Hyoscine Butylbromide with 10 ml of *0.001M hydrochloric acid* with the aid of ultrasound for 15 minutes, centrifuge and filter.

Reference solution (a). A 0.001 per cent w/v solution of *hyoscine hydrobromide IPRS* in *0.001 M hydrochloric acid.*

Reference solution (b). Add 10 µl of the test solution to 10 ml of reference solution (a).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (10 µm),
- mobile phase: dissolve 2.0 g of *sodium dodecyl sulphate* in a mixture of 370 ml of *0.001M hydrochloric acid* and 680 ml of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to hyoscine and butylhyoscine is not less than 5.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to hyoscine is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254.*

Mobile phase. A mixture of 0.5 volume of *anhydrous formic acid* , 1.5 volumes of *water*, 9 volumes of *ethanol* and 9 volumes of *methylene chloride*.

Test solution. Shake a quantity of the powdered tablets containing 20 mg of Hyoscine Butylbromide with 5 ml of *0.01M hydrochloric acid* and filter.

Reference solution (a). Dilute 3 volumes of the test solution to 100 volumes with *0.01M hydrochloric acid.*

Reference solution (b). Dilute 1 volume of the test solution to 50 volumes with *0.01M hydrochloric acid.*

Reference solution (c). Dilute 1 volume of the test solution to 400 volumes with *0.01M hydrochloric acid.*

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 4 cm, dry the plate at 60° for 15 minutes and spray with a solution prepared by mixing equal volumes of a 40 per cent w/v solution of *potassium iodide* in *water* and a solution prepared by dissolving 0.85 g of *bismuth oxynitrate* in a mixture of 10 ml of *glacial acetic acid* and 40 ml of *water* and diluting 1 volume of the mixture with 2 volumes of *glacial acetic acid* and 10 volumes of *water* immediately before use. Allow the plate to dry in air, spray well with 5.0 per cent w/v solution of *sodium nitrite* and examine immediately.

In the chromatogram obtained with the test solution, the principal spot has an R_f value of 0.45.

In the chromatogram obtained with the test solution any secondary spot with an R_f value less than that of the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (3.0 per cent), not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent); any secondary spot with an R_f value more than that of the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (2.0 per cent) and not more than one such spot with an R_f value more than that of the principal spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using following test solution.

Test solution. Disperse one tablet in 25.0 ml of *0.001 M hydrochloric acid* with the aid of ultrasound.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 40 mg of Hyoscine Butylbromide in 60 ml of *0.001M hydrochloric acid*, dilute to 100.0 ml with *0.001M hydrochloric acid*, centrifuge and filter.

Reference solution. A 0.04 per cent w/v of solution of *hyoscine butylbromide IPRS* in *0.001M hydrochloric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (10 µm) (Such as Lichrosorb C8),
- mobile phase: a buffer solution prepared by dissolving 2.0 g *sodium lauryl sulphate* in a mixture of 370 volumes of *0.001 M hydrochloric acid* and 680 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0 per cent and the relative standard deviation for replicate injections is not more than 2.0.

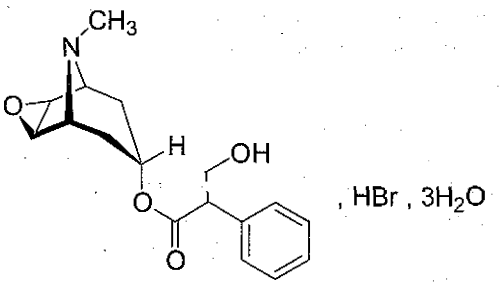
Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{30}BrNO_4$ in the tablets.

Storage. Store at a temperature not exceeding 30° protected from light and moisture.

Hyoscine Hydrobromide

Scopolamine Hydrobromide



$C_{17}H_{21}NO_4,HBr,3H_2O$ Mol. Wt. 438.3

Hyoscine Hydrobromide is (1*S*,3*s*,5*R*,6*R*,7*S*)-6,7-epoxytropan-3-yl(*S*)-tropate hydrobromide trihydrate.

Hyoscine Hydrobromide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{21}NO_4,HBr$, calculated on the anhydrous basis.

Category. Parasympatholytic.

Description. Colourless crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hyoscine hydrobromide IPRS* or with the reference spectrum of hyoscine hydrobromide.

B. To about 1 mg add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 2 ml of *acetone* and add 0.1 ml of a 3 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour is produced.

C. It gives the reaction of alkaloids (2.3.1).

D. It gives the reactions of bromides (2.3.1).

Tests

pH (2.4.24). 4.0 to 5.5, determined in a 5.0 per cent w/v solution.

Specific optical rotation (2.4.22). –27.0° to –24.0°, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 70 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with mobile phase.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 25.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.001 per cent w/v of *hyoscine hydrobromide impurity B IPRS* and 0.0014 per cent w/v of the substance under examination in the mobile phase.

Chromatographic system

- stainless steel column 12.5 cm x 4.0 mm, packed with octylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 330 volumes of *acetonitrile* and 670 volumes of a 0.25 per cent w/v solution of *sodium dodecyl sulphate*, previously adjusted to pH 2.5 with *3 M orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 5 µl.

Name	Relative retention time	Correction factor
Hyoscine impurity D[1]	0.2	0.3
Hyoscine impurity B[2]	0.9	—
Hyoscine (Retention time: about 5 minutes)	1.0	—
Hyoscine impurity A[3]	1.3	—
Hyoscine impurity C[4]	2.4	0.6

[1]DL-tropic acid,

[2]norhyoscine,

[3]hyoscyamine,

[4]apohyoscine.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to hyoscine impurity B and hyoscine is not less than 1.5 and the tailing factor for the peak due to hyoscine is not more than 2.5.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the peak due to hyoscine. In the chromatogram obtained with the test solution, the area of peak corresponding to hyoscine impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of peak corresponding to hyoscine impurities A, C and D is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of areas of all the secondary peaks is not more than 1.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) and the peak due to bromide ion which appears close to the solvent peak.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 10.0 to 13.0 per cent, determined on 0.2 g.

Assay. Weigh 0.4 g, dissolve in 10 ml of *anhydrous glacial acetic acid*, warming if necessary, cool the solution and add 20 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03843 g of $C_{17}H_{21}NO_4,HBr$.

Storage. Store protected from light and moisture in well-filled containers of small capacity in a refrigerator at a temperature not exceeding 15°.

Hyoscine Hydrobromide Injection

Scopolamine Hydrobromide Injection

Hyoscine Hydrobromide Injection is a sterile solution of Hyoscine Hydrobromide in Water for Injection.

Hyoscine Hydrobromide Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscine hydrobromide, $C_{17}H_{21}NO_4,HBr,3H_2O$.

Usual strength. 400 µg per ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*.

Test solution. Evaporate a volume of the injection containing 5 mg of Hyoscine Hydrobromide to dryness on a water-bath, triturate the residue with 1 ml of *ethanol (95 per cent)*, allow to stand and use the supernatant liquid.

Reference solution. A solution containing 0.5 per cent w/v of *hyoscine hydrobromide IPRS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, heat it at 105° for 20 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the chromatogram obtained with test solution (b) shows a peak with the same retention time as the peak derived from hyoscine hydrobromide in the chromatogram obtained with the reference solution.

C. Evaporate a suitable volume to dryness. To 1 ml of a 1.0 per cent w/v solution of the residue add 1 ml of *5 M ammonia*, shake with *chloroform* and evaporate the chloroform solution to dryness on a water-bath. To the residue add 1.5 ml of a 2.0 per cent w/v solution of *mercuric chloride* in *ethanol (60 per cent)*; a white precipitate is produced which dissolves on warming (distinction from atropine and hyoscyamine).

D. It gives reaction (A) of bromides (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.0.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing about 0.04 g of Hyoscine Hydrobromide in 100.0 ml of *water*.

Reference solution. A 0.04 per cent w/v solution of *hyoscine hydrobromide IPRS* in *water*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Lichrosorb RP18),
- mobile phase: a mixture of 1 volume of 60 per cent w/v solution of *perchloric acid*, 31 volumes of *methanol* and 68 volumes of *water*, adjusted to pH 2.5 with *13.5 M ammonia*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ in the injection.

1 mg of $C_{17}H_{21}NO_4,HBr$ is equivalent to 1.141 mg of $C_{17}H_{21}NO_4,HBr,3H_2O$.

Storage. Store at a temperature not exceeding 15°, protected from light.

Hyoscine Hydrobromide Tablets

Scopolamine Hydrobromide Tablets

Hyoscine Hydrobromide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscine hydrobromide, $C_{17}H_{21}NO_4,HBr,3H_2O$.

Usual strengths. 300 µg; 600 µg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 40 volumes of *acetone* and 10 volumes of *diethylamine*.

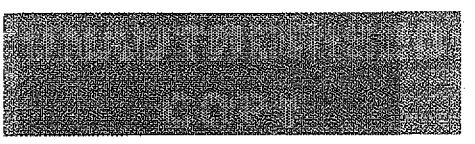
Test solution. Shake a quantity of the powdered tablets containing 10 mg of Hyoscine Hydrobromide with 2 ml of *ethanol (95 per cent)* and centrifuge.

Reference solution. A solution containing 0.5 per cent w/v of *hyoscine hydrobromide IPRS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, heat it at 105° for 20 minutes, allow to cool and spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the chromatogram obtained with test solution (b) shows a peak with the same retention time as the peak derived from hyoscine hydrobromide in the chromatogram obtained with the reference solution.

C. Extract a quantity of the powdered tablets containing 1 mg of Hyoscine Hydrobromide with 5 ml of *ethanol (95 per cent)*, filter and evaporate the filtrate to dryness on a water-bath. Cool, add 0.2 ml of *fuming nitric acid* and again evaporate to dryness on a water-bath; a yellow residue is produced. To the cooled residue add 2 ml of *acetone* and 0.2 ml of a 3.0 per cent w/v solution of *potassium hydroxide* in *methanol*; a deep violet colour is produced. (Atropine and hyoscyamine also yield this reaction; the reaction is masked by other alkaloids).



D. The powdered tablets give reaction (A) of bromides (2.3.1).

Tests

Uniformity of content. Complies with the test stated under Tablets.

For tablets containing 600 µg of Hyoscine Hydrobromide—

Test solution (a). Powder one tablet and triturate with 5 ml of *0.1 M hydrochloric acid.* Add 1.0 ml of a 0.0375 per cent w/v solution of *atropine sulphate IPRS* (internal standard) in *methanol* (solution A), extract with two quantities, each of 5 ml, of *chloroform* and discard the chloroform extracts. Add 1 ml of *5 M ammonia.* Extract with two quantities, each of 5 ml, of *chloroform*, shake the combined extracts with 1 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in 0.5 ml of a mixture of 20 volumes of *dichloromethane*, 4 volumes of *N,O-bis(trimethylsilyl) acetamide* and 1 volume of *trimethylchlorosilane*, mix and allow to stand for 30 minutes.

Test solution (b). Prepare in the same manner as test solution (a) but omitting the addition of solution A.

Reference solution. Add 1 ml of a 0.0375 per cent w/v solution of *atropine sulphate IPRS* (internal standard) in *methanol* (solution A) and 1 ml of *5 M ammonia* to 5.0 ml of a 0.012 per cent w/v solution of *hyoscine hydrobromide IPRS* and complete the procedure described under test solution (a) beginning at the words "extract with two quantities,...".

Carry out the procedure as described under Assay. Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ in the tablet.

For tablets containing less than 600 µg of Hyoscine Hydrobromide — Use the same procedure but with correspondingly smaller concentrations of *hyoscine hydrobromide IPRS* and *atropine sulphate IPRS.*

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of *water.*

Internal standard solution. A 0.02 per cent w/v solution of *atropine sulphate IPRS* in the solvent mixture.

Test solution (a). Disperse 10 whole tablets in 7 ml of the solvent mixture with the aid of ultrasound and dilute to obtain a solution containing 0.015 per cent w/v of Hyoscine Hydrobromide in the solvent mixture, centrifuge and filter the supernatant liquid.

Test solution (b). Disperse 10 whole tablets in 7 ml of the solvent mixture with the aid of ultrasound and dilute to obtain a solution containing 0.015 per cent w/v of Hyoscine Hydrobromide in internal standard solution.

Reference solution. A 0.015 per cent w/v solution of *hyoscine hydrobromide IPRS* in internal standard solution.

Chromatographic system

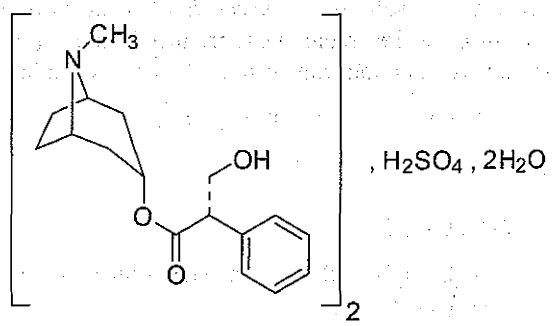
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Lichrosorb RP18),
- mobile phase: *0.05 M sodium octanesulphonate* in a mixture of 1 volume of a 60 per cent w/v solution of *perchloric acid*, 3 volumes of *methanol*, 21 volumes of *acetonitrile* and 75 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject the reference solution, internal standard solution, test solution (a) and (b).

Calculate the content of $C_{17}H_{21}NO_4,HBr,3H_2O$ in the tablets. 1 mg of $C_{17}H_{21}NO_4,HBr$ is equivalent to 1.141 mg of $C_{17}H_{21}NO_4,HBr,3H_2O$.

Storage. Store at a temperature not exceeding 15°, protected from light.

Hyoscyamine Sulphate



$(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$ Mol. Wt. 712.9

Hyoscyamine Sulphate is bis (1*R*,3*r*,5*S*)- 8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropionate] sulphate dihydrate.

Hyoscyamine Sulphate contains not less than 98.0 per cent and not more than 101.0 per cent of $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$, calculated on the anhydrous basis.

Category. Anticholinergic.

Description. A white or almost white, crystalline powder or colourless needles.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *hyoscyamine sulphate IPRS* or with the reference spectrum of hyoscyamine sulphate.

B. To 0.5 ml of solution A, add 2 ml of *dilute acetic acid* and heat. To the hot solution add 4 ml of *picric acid solution*, allow to cool, shaking occasionally. Collect the crystals, wash with 2 quantities, each of 3 ml, of iced *water* and dry at 105°. The crystal melts between 164° to 168°(2.4.21).

C. To about 1 mg of test solution add 0.2 ml of *fuming nitric acid* and evaporate to dryness on a water-bath and dissolve the residue in 2 ml of *acetone*, add 0.2 ml of a 3.0 per cent w/v solution of *potassium hydroxide* in *methanol*; a violet colour develops.

D. It gives reaction (A) of sulphates (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *water* (solution A) is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 4.5 to 6.2, determined in 2.0 per cent w/v solution in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). –29.0° to –24.0°, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 60 mg of the substance under examination to 50.0 ml with mobile phase A. Further dilute 10.0 ml of the solution to 50.0 ml with mobile phase A.

Reference solution (a). Dilute 5.0 ml of the test solution to 100.0 ml with mobile phase A. Further dilute 5.0 ml of the solution to 50.0 ml with mobile phase A.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 25.0 ml with mobile phase A.

Reference solution (c). Dissolve 5 mg of *(1R,3r,5S)-8-azabicyclo[3.2.1]oct-3-yl(2S)-3-hydroxy-2-phenylpropanoate(norhyoscyamine) IPRS (hyoscyamine impurity E IPRS)* in the 20.0 ml of test solution. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature. 25°,
- mobile phase: A. dissolve 3.5 g of *sodium dodecyl sulphate* in 606 ml of a 0.7 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted to pH 3.3 with *0.05 M orthophosphoric acid* and mix with 320 ml of *acetonitrile*,

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
2	95	5
20	70	30
20.1	95	5
25	95	5

Inject reference solution (c). The relative retention time for (2*RS*)-3-hydroxy-2-phenylpropanoic acid (DL-tropic acid) (hyoscyamine impurity A) is about 0.2, for (1*R*,3*S*,5*R*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate(7-hydroxyhyoscyamine) (hyoscyamine impurity B) is about 0.67, for (1*S*,3*R*,5*S*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate (6-hydroxyhyoscyamine) (hyoscyamine impurity C) is about 0.72, hyoscine (hyoscyamine impurity D) is about 0.8, for (1*R*,3*r*,5*S*)-8- azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate(norhyoscyamine) (hyoscyamine impurity E) is about 0.9, for (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*R*)-2-hydroxy-3-phenyl propanoate (littorine) (hyoscyamine impurity F) is about 1.1 and for (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-phenylprop-2-enoate (apoatropine) (hyoscyamine impurity G) is about 1.8. The test is not valid unless resolution between the peaks due to hyoscyamine and hyoscyamine impurity E is not less than 2.5. Multiply the peak areas of the impurities by the correction factor for calculating the contents, for impurity A is 0.3; impurity G is 0.6.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak corresponding to impurity E is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of the secondary peaks corresponding to hyoscyamine impurities A, B, C, D, F, G, for each impurity, is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all secondary peaks is not more than the area of the principal peak in the

chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 2.0 to 5.5 per cent, determined on 0.5 g.

Assay. Weigh 0.5 g and dissolve in 25 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0677 g of $C_{34}H_{48}N_2O_{10}S$.

Storage. Store protected from light and moisture.

Hyoscyamine Injection

Hyoscyamine Sulphate Injection

Hyoscyamine Injection is a sterile solution of Hyoscyamine Sulphate in Water for Injections.

Hyoscyamine Injection contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of hyoscyamine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$.

Usual strengths. 0.5 mg per ml; 2.0 mg per ml.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

B. After evaporation to dryness, complies with the test for sulphates (2.3.1).

Tests

pH (2.4.24). 3.0 to 6.5.

Bacterial Endotoxin test (2.2.3). Not more than 714.3 Endotoxin Units per mg of hyoscyamine sulphate.

Other tests. Comply with the tests stated under Parenteral Preparations (Injection).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing about 1 mg of Hyoscyamine Sulphate to 200.0 ml with *0.01 M hydrochloric acid.*

Reference solution (a). A 0.016 per cent w/v solution of *hyoscyamine sulphate IPRS* in *0.01 M hydrochloric acid.*

Reference solution (b). Dilute 3.0 ml of reference solution (a) to 100.0 ml with *0.01 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 1800 volumes of the buffer solution, prepared by dissolving 13.6 g of *monobasic potassium phosphate* in 1800 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, dilute to 2000 ml with *water*, add 0.3 volume of *triethylamine* and 200 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 50 µl.

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$ in the injection.

Storage. Store in single-dose or multiple-dose containers, preferably of Type I glass at controlled room temperature.

Hyoscyamine Oral Solution

Hyoscyamine Sulphate Oral Solution

Hyoscyamine Oral Solution is a mixture consisting of Hyoscyamine sulphate with buffering agents and other excipients. It contains suitable flavouring agents. It is filled in a sealed container.

The oral solution is constituted by dispersing the contents of the sealed container in the specified volume of water just before use.

Hyoscyamine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscyamine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$.

Usual strength. 0.125 mg per ml.

Storage. Store the constituted solution in a refrigerator 2° to 8°. Discard any unused portion after 30 days of reconstitution.

The contents of the sealed container comply with the requirements stated under Oral Liquids and with the following requirements.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

pH (2.4.24). 3.0 to 6.5, determined in the reconstituted solution.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the constituted solution containing 0.5 mg of Hyoscyamine Sulphate and dissolve in 100.0 ml of *0.01 M hydrochloric acid.*

Reference solution (a). A 0.016 per cent w/v solution of *hyoscyamine sulphate IPRS* in *0.01 M hydrochloric acid.*

Reference solution (b). Dilute 3.0 ml of reference solution (a) to 100.0 ml with *0.01 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of the buffer solution prepared by dissolving 13.6 g of *monobasic potassium phosphate* in 1800 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*, dilute to 2000 ml with *water*, add 0.3 volume of *triethylamine* and 10 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 50 µl.

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 1.8 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$ in oral solution.

Hyoscyamine Tablets

Hyoscyamine Sulphate Tablets

Hyoscyamine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of hyoscyamine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$.

Usual strength. 0.125 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

B. It gives reaction (A) of sulphates (2.3.1).

Tests

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

Test solution. Disperse 1 Tablet in 25 ml of *0.01 M hydrochloric acid.*

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 Tablets. Disperse a quantity of powder containing about 0.125 mg of Hyoscyamine Sulphate in 25.0 ml of *0.01 M hydrochloric acid.*

Reference solution (a). A 0.016 per cent w/v solution of *hyoscyamine sulphate IPRS* in *0.01 M hydrochloric acid.*

Reference solution (b). Dilute 3.0 ml of reference solution (a) to 100 ml with *0.01 M hydrochloric acid.*

Tropic acid solution. A 0.0003 per cent w/v solution of tropic acid in *0.01 M hydrochloric acid.*

Reference solution (c). To 3.0 ml of the reference solution (a), add 4.0 ml of the tropic acid solution and dilute to 100 ml with *0.01 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with phenyl groups bonded to porous silica (4 µm),
- mobile phase: a mixture of 0.3 volume of *triethylamine,* 1800 volumes of the buffer solution prepared by dissolving about 13.6 g of *monobasic potassium phosphate* in 1800 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid,* dilute to 2000 ml with *water* and 200 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 50 µl.

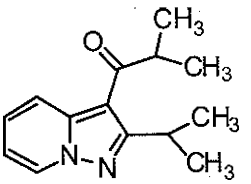
Inject reference solution (c). The test is not valid unless the resolution between the peaks due to hyoscyamine and tropic acid is not less than 1.5 and the tailing factor is not more than 1.8. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $(C_{17}H_{23}NO_3)_2,H_2SO_4,2H_2O$ in the tablets.

I

Ibudilast



$C_{14}H_{18}N_2O$ Mol Wt. 230.3

Ibudilast is 2-Methyl-1-(2-propan-2-ylpyrazolo[1,5-a]pyridin-3-yl)propan-1-one.

Ibudilast contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{18}N_2O$, calculated on the dried basis.

Category. Antiallergic; antiasthamatic and vasodilator.

Description. A white to off white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ibudilast IPRS* or with the reference spectrum of ibudilast.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and diluted to 50 .0 ml with the mobile phase.

Reference solution. A 0.05 per cent w/v solution of *ibudilast IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *methanol* and 20 volumes of *water*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 3.0.

Inject test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks peaks is not more than 1.5 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 43° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50 .0 ml with the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. A 0.0025 per cent w/v solution of *ibudilast IPRS* in the mobile phase.

Chromatographic system

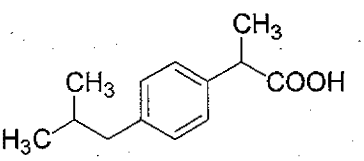
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *methanol* and 20 volumes of *water*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{18}N_2O$

Ibuprofen



$C_{13}H_{18}O_2$ Mol. Wt. 206.3

Ibuprofen is (*RS*)-2-(4-isobutylphenyl)propionic acid.

Ibuprofen contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{13}H_{18}O_2$, calculated on the dried basis.

Category. Anti-inflammatory; analgesic.

Description. A white or almost white, crystalline powder or colourless crystals.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ibuprofen IPRS* or with the reference spectrum of ibuprofen.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at about 264 nm and 272 nm, and a shoulder at about 258 nm. The ratio of the absorbance at about 264 nm to that at the shoulder at about 258 nm is 1.20 to 1.30. The ratio of the absorbance at the maximum at about 272 nm to that at the shoulder at about 258 nm is 1.00 to 1.10.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 75 volumes of *n-hexane*, 25 volumes of *ethyl acetate* and 5 volumes of *glacial acetic acid*.

Test solution. Dissolve 0.5 g of the substance under examination in 100.0 ml of *dichloromethane*.

Reference solution. A 0.5 per cent w/v solution of *ibuprofen IPRS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 120° for 30 minutes, lightly spray the plate with a 1 per cent w/v solution of *potassium permanganate* in *1 M sulphuric acid*, heat at 120° for 20 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

Optical rotation (2.4.22). – 0.05° to + 0.05°, determined in a 2.5 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 2 ml of *acetonitrile* and add sufficient of the mobile phase to produce 10.0 ml.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 20 mg of *ibuprofen IPRS* in 2 ml of *acetonitrile*, add 1 ml of 0.006 per cent w/v solution of *2-(4-butylphenyl)propionic acid IPRS* in *acetonitrile* and add sufficient mobile phase to produce 10.0 ml.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 600 volumes of *water*, 340 volumes of *acetonitrile* and 0.5 volume of *orthophosphoric acid* diluted to 1000 volumes with *water* after equilibration,
- flow rate: 2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 45 minutes before starting the chromatography.

Inject reference solution (a) and (b). In the chromatogram obtained with reference solution (b) measure the height (*a*) of the peak due to 2-(4-butylphenyl) propionic acid and the height (*b*) of the lowest point of the curve separating this peak from that due to ibuprofen. The test is not valid unless *a* is greater than 1.5*b*. If necessary, adjust the concentration of acetonitrile in the mobile phase to obtain the required resolution. Verify the repeatability by making five separate injections of 20 µl of reference solution (a). The test is not valid unless the relative standard deviation of the area of the principal peak is less than 2.0 per cent.

Inject reference solution (a), (b) and the test solution. Run the chromatogram for 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-(4-butylphenyl) propionic acid is not greater than that of the peak due to 2-(4-butylphenyl) propionic acid in the chromatogram obtained with reference solution (b), the area of any other secondary peak is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) and the sum of the areas of any such peaks is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a).

Impurity F. Determine by gas chromatography (2.4.13).

Methylating solution. Dilute 1 ml of *N,N-dimethylformamide dimethyl acetal* and 1 ml of *pyridine* to 10 ml with *ethyl acetate*.

Test solution. Weigh about 50 mg of the substance under examination into a sealable vial, dissolve in 1.0 ml of *ethyl acetate*, add 1 ml of the methylating solution, seal and heat at 100° in a block heater for 20 minutes, cool. Remove the reagents under a stream of nitrogen at room temperature. Dissolve the residue in 5 ml of *ethyl acetate*.

Reference solution (a) Dissolve 0.5 mg of *ibuprofen impurity F IPRS* in *ethyl acetate* and dilute to 10.0 ml with *ethyl acetate*.

Reference solution (b) Weigh about 50.0 mg of *ibuprofen IPRS* into a sealable vial, dissolve in 1.0 ml of reference solution (a), add 1 ml of the methylating solution, seal and heat at 100° in a block heater for 20 min. Allow to cool. Remove the reagents under a stream of nitrogen at room temperature. Dissolve the residue in 5 ml of *ethyl acetate*.

Chromatographic system
- a capillary column 25 m x 0.53 mm, packed with fused silica coated with macrogol 20000 (film thickness 2 µm),
- temperature:
  column 150°,
  injector port: 200° and detector port at 250°,
- flame ionization detector,
- flow rate: 5 ml per minute using nitrogen as the carrier gas.

Inject 1 µl of reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. The relative retention time with reference to ibuprofen for 3-[4-(2methylpropyl)phenyl] propionic acid (ibuprofen impurity F) is about 1.5. The area of the peak corresponding to ibuprofen impurity F is not more than 0.1 per cent of the principal peak.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.1 kPa.

Assay. Weigh 0.4 g, dissolve in 100 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02063 g of $C_{13}H_{18}O_2$.

Ibuprofen Cream

Ibuprofen Cream contains Ibuprofen in a suitable cream base.

Ibuprofen Cream contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ibuprofen, $C_{13}H_{18}O_2$.

Usual strength. 15 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 5 volumes of *anhydrous acetic acid*, 25 volumes of *ethyl acetate* and 75 volumes of *n-hexane*.

Test solution. Shake a quantity of the cream containing 50 mg of Ibuprofen with 10 ml of *dichloromethane* for 5 minutes and filter.

Reference solution. A 0.5 per cent w/v solution of *ibuprofen IPRS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 120° for 30 minutes, lightly spray the plate with a 1 per cent w/v solution of *potassium permanganate* in *1M sulphuric acid*, heat at 120° for 20 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the cream containing 0.1 g of Ibuprofen with 25 ml of *methanol* for 10 minutes, dilute to 50 ml with *methanol* and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*.

Reference solution (b). Dissolve 50 mg of *ibuprofen IPRS* in 2.5 ml of a 0.006 per cent w/v solution of *2-(4-butylphenyl) propionic acid IPRS* in *methanol* and dilute to 25.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 2),
- mobile phase: a mixture of 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile* and 660 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject reference solution (b). Measure the height (a) of the peak due to 2-(4-butylphenyl)-propionic acid and the height (b) of the lowest point of the curve separating this peak from that due to ibuprofen. The test is not valid unless (a) is more than 1.5 (b).

Inject reference solution (a), (b) and the test solution. Run the chromatogram for 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-(4-butylphenyl)-propionic acid is not more than that of the peak due to 2-(4-butylphenyl)propionic acid in the chromatogram obtained with reference solution (b), the area of any other secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a)

(0.3 per cent) and the sum of the areas of other secondary peaks is not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the cream containing 50 mg of ibuprofen with 25 ml of the mobile phase for 10 minutes, decant the solution into a 50 ml graduated flask, rinse the original flask with two 10 ml quantities of the mobile phase, dilute the combined solution and rinsings to 50 ml with the mobile phase and filter.

Reference solution. A 0.1 per cent w/v solution of *ibuprofen IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (10 µm) (such as Nucleosil C18),
- mobile phase: a mixture of 0.3 volumes of *orthophosphoric acid*, 24.7 volumes of *water* and 75 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 264 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{18}O_2$ in the cream.

Ibuprofen Gel

Ibuprofen Gel is a solution of Ibuprofen in a suitable water-miscible base.

Ibuprofen Gel contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ibuprofen, $C_{13}H_{18}O_2$.

Usual strength. 15 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 5 volumes of *anhydrous acetic acid*, 25 volumes of *ethyl acetate* and 75 volumes of *n-hexane*.

Test solution. Weigh a quantity containing 50 mg of Ibuprofen and transfer to a separating funnel with the aid of 10 ml of *dichloromethane*. Shake vigorously for 5 minutes and filter.

Reference solution. A 0.5 per cent w/v solution of *ibuprofen IPRS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. After development, dry the plate at 120° for 30 minutes, lightly spray the plate with a 1 per cent w/v solution of *potassium permanganate* in *1 M sulphuric acid*, heat at 120° for 20 minutes and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the substance under examination containing 0.1 g of Ibuprofen in 25 ml of warm *methanol*, cool and dilute to 50.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*.

Reference solution (b). Dissolve 50 mg of *ibuprofen IPRS* in 2.5 ml of a 0.006 per cent w/v solution of *2-(4-butylphenyl)-propionic acid IPRS* in *methanol* and dilute to 25.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.5 volume of *orthophosphoric acid*, 340 volumes of *acetonitrile* and 660 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for 45 minutes.

Inject reference solution (b). Measure the height (a) of the peak due to 2-(4-butylphenyl) propionic acid and the height (b) of the lowest point of the curve separating this peak from that due to ibuprofen.

The test is not valid unless *a* is greater than 1.5*b*. If necessary, adjust the concentration of acetonitrile in the mobile phase to obtain the required resolution.

Inject reference solution (a), (b) and the test solution. Run the chromatogram for 1.5 times the retention time of the principal

peak. In the chromatogram obtained with the test solution the area of any peak corresponding to 2-(4-butylphenyl) propionic acid is not more than that of the peak due to 2-(4- butylphenyl)-propionic acid in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of the areas of other secondary peaks is not more than 0.7 times the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.7 per cent). Ignore any peak the area of which is less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Gel.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the substance under examination containing 50 mg of Ibuprofen with 50 ml of warm *methanol* for 10 minutes, cool and add sufficient *methanol* to produce 100 ml. Dilute 10.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution. Dilute 10.0 ml of a solution containing 0.05 per cent w/v of *ibuprofen IPRS* in *methanol* to 20.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 3 volumes of *ortho-phosphoric acid*, 247 volumes of *water* and 750 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 264 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{18}O_2$ in the gel.

Ibuprofen Tablets

Ibuprofen Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ibuprofen, $C_{13}H_{18}O_2$. The tablets are coated.

Usual strengths. 200 mg; 400 mg; 600 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.5 g of Ibuprofen with 20 ml of *acetone*, filter and evaporate the filtrate to dryness in a current of air without heating. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ibuprofen IPRS* or with the reference spectrum of ibuprofen.

B. The residue obtained in test A, after recrystallisation from *light petroleum (40° to 60°)*, melts at about 75° (2.4.21).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 7.2*,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 221 nm (2.4.7). Similarly, measure the absorbance of the solution of known concentration of *ibuprofen IPRS*. Calculate the content of $C_{13}H_{18}O_2$.

Q. Not less than 75 per cent of the stated amount of $C_{13}H_{18}O_2$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 75 volumes of *n-hexane*, 25 volumes of *ethyl acetate* and 5 volumes of *glacial acetic acid*.

Test solution. Extract a quantity of the powdered tablets containing 0.2 g of Ibuprofen with three quantities, each of 10 ml, of *chloroform*, filter, evaporate the combined filtrate to about 1 ml and add sufficient *chloroform* to produce 2 ml.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, lightly spray the plate with a 1 per cent w/v solution of *potassium permanganate* in *1 M sulphuric acid*, heat at 120° for 20 minutes and examine under ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any spot with an R_f value relative to ibuprofen of about 1.2.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.5 g of Ibuprofen, extract with 60 ml of *chloroform* for 15 minutes and filter through a sintered-glass crucible of porosity 3. Wash the residue with three quantities, each of 10 ml, of *chloroform* and gently evaporate the filtrate just to dryness in a current of air. Dissolve the

residue in 100 ml of *ethanol (95 per cent)*, previously neutralized to *phenolphthalein solution*, and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02063 g of $C_{13}H_{18}O_2$.

Ibuprofen and Paracetamol Tablets

Ibuprofen and Paracetamol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ibuprofen, $C_{13}H_{18}O_2$ and paracetamol, $C_8H_9O_2$.

Usual strength. Ibuprofen, 400 mg and Paracetamol, 325 mg.

Identification

In the Assay, the two principal peaks in the chromatogram obtained with the test solution correspond to the peaks due to ibuprofen and paracetamol in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium.

For ibuprofen — 900 ml of phosphate buffer pH 7.2.

For paracetamol — 900 ml of phosphate buffer pH 5.8.

Speed and time. 50 rpm and 45 minutes,

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve an accurately weighed quantity of *ibuprofen IPRS* and *paracetamol IPRS* in minimum quantity of *acetonitrile* and dilute with dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 3.9 g of *sodium di-hydrogen phosphate dihydrate* and 8.9 g of *disodium hydrogen phosphate dihydrate* in *water*, adjusted to pH 7.0 with *orthophosphoric acid*, diluted to 1000 ml with *water* and 25 volumes of *acetonitrile*.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections for each of the peaks corresponding to paracetamol and ibuprofen is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of paracetamol, $C_8H_9O_2$ and ibuprofen, $C_{13}H_{18}O_2$.

Q. Not less than 75 per cent of the stated amount of paracetamol, $C_8H_9O_2$ and ibuprofen, $C_{13}H_{18}O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solution A. Dissolve 6.8 g of *potassium dihydrogen phosphate* in 1000 ml of *water* and adjusted to pH 3.0 with *orthophosphoric acid.*

Solvent mixture. A mixture of 40 volumes of solution A and 60 volumes of *methanol.*

Test solution. Weigh and transfer a quantity of powdered tablets containing 0.1 g of ibuprofen to a 50.0 ml volumetric flask, add 30.0 ml of solvent mixture and disperse with the aid of ultrasound for 10 minutes. Cool and dilute to volume with solvent mixture, and filter, rejecting the first few ml of the filterate.

Reference solution (a). Dissolve about 40 mg of *ibuprofen IPRS* and 33 mg of *paracetamol IPRS* in 60 ml of solvent mixture with intermittent shaking. Dilute to 200.0 ml with solvent mixture.

Reference solution (b). Dilute 3.0 ml of reference solution (a) to 100.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 25°,
- auto sampler temperature: 10°,
- mobile phase: A. a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and add 0.25 g *hexane-1-sulphonic acid sodium salt* and filter,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 10 µl,

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
6	98	2
16	80	20
22	65	35
55	60	40
63	45	55
63.1	98	2
70	98	2

Name	Relative retention time
p-Amino Phenol	0.36
Paracetamol	1.0
p-Nitro Phenol	2.95
p-Chloroacetanilide	3.21
Ibuprofen Impurity J[1]	3.77
Ibuprofen Impurity A[2]	5.86
Ibuprofen	6.0
Ibuprofen Impurity B[3]	6.23

[1] (2*RS*)-2-[4-(2-methylpropanoyl)phenyl]propanoic acid,

[2] (2*RS*)-2-[3-(2-methylpropyl)phenyl]propanoic acid,

[3] (2*RS*)-2-(4-butylphenyl)propanoic acid.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 for both the principal peaks.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to p-amino phenol, p-nitro phenol, p-chloroacetanilide and ibuprofen impurity J, A, B are not more than the area of the principal peak of paracetamol and ibuprofen, respectively in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak of paracetamol in the chromatogram obtained with reference solution (b) (0.3 per cent) and the sum of areas of all secondary peaks is not more than 6.66 times the area of the principal peak of paracetamol in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 160 mg of Ibuprofen to 100.0 ml with mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the same solvent.

Reference solution. A solution containing 0.013 per cent w/v of *paracetamol IPRS* and 0.016 per cent of *ibuprofen IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 3.9 g of *sodium di-hydrogen phosphate dihydrate* and 8.9 g of *disodium hydrogen phosphate dihydrate* in *water*, adjusted to pH 7.0 with *orthophosphoric acid*, diluted to 1000 ml with *water* and 24.5 volumes of *acetonitrile* and 0.5 volume of *methanol*.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the resolution between the two principal peaks is not less than 10.0 and the relative standard deviation for replicate injections for both the principal peaks are not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of paracetamol, $C_8H_9O_2$ and ibuprofen, $C_{13}H_{18}O_2$, in the tablets.

Storage. Store protected from moisture, at a temperature below 30°.

Ibuprofen and Pseudoephedrine Hydrochloride Tablets

Ibuprofen and Pseudoephedrine Hydrochloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ibuprofen, $C_{13}H_{18}O_2$ and pseudoephedrine hydrochloride, $C_{10}H_{15}NO,HCl$.

Usual strength. Pseudoephedrine Hydrochloride, 30 mg and Ibuprofen, 200 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *chloroform*, 15 volumes of *methanol* and 5 volumes of *glacial acetic acid*.

Test solution. Shake a quantity of the powdered tablets containing 30 mg of Pseudoephedrine Hydrochloride in 10 ml of *methanol*, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.2 per cent w/v solution of *pseudoehedrine hydrochloride IPRS* in *methanol*.

*Reference solution (b).*A 0.2 per cent w/v solution of *ibuprofen IPRS* in *methanol.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 5 to 10 minutes and expose to iodine vapour for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and (b).

B.In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (d).

Tests

Dissolution (2.5.2).

For Ibuprofen —

Apparatus No. 2 (Paddle),
Medium: 900 ml of *phosphate buffer pH 7.2*,
Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 224 nm (2.4.7). Calculate the content of ibuprofen, $C_{13}H_{18}O_2$ in the medium from the absorbance obtained from a solution of known concentration of *ibuprofen IPRS.*

Q. Not less than 75 per cent of the stated amount of $C_{13}H_{18}O_2$.

For Pseudoephedrine hydrochloride —

Apparatus No. 2 (Paddle),

Medium: 900 ml of *phosphate buffer pH 7.2*,
Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *pseudoephedrine hydrochloride IPRS* in the dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with silica chemically-bonded nitrile groups (5 µm),
- mobile phase: a mixture of equal volumes of a buffer solution prepared by dissolving 0.5 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 3.3 with *ortho phosphoric acid* and *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per centand the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{15}NO,HCl$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{10}H_{15}NO,HCl$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

*Test solution.*Weigh and powder 20 tablets. Disperse a quantity of the powder containing 200 mg of Ibuprofen in 20.0 ml volumetric flask, add equal volume of reference solution (a) and *acetonitrile,* sonicate and filter.

*Reference solution(a).*A 0.015 per cent w/v solution of *butylparaben* in mobile phase.

*Reference solution(b).*A 2.0 per cent w/v solution of *ibuprofen IPRS* in equal volume of reference solution (a) and *acetonitrile.*

*Reference solution(c).*A 0.3 per cent w/v solution of *pseudoephedrine hydrochloride IPRS* in equal volume of reference solution (a) and *acetonitrile.*

*Reference solution(d).*Dilute reference solution (b) and (c) with the *acetonitrile* to obtain a solution having a known concentration similar to the test solution.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a buffer solution prepared by dissolving 2.5 g of *docusate sodium* in a mixture of 50 volumes of *water* and 41 volumes of *acetonitrile* and add 1.0 ml of *orthophosphoric acid*, adjusted to pH 3.2 with *ammonium hydroxide.*
- flow rate: 2 ml per minute,
- spectrophotometer set at 254nm,
- injection volume: 5 µl.

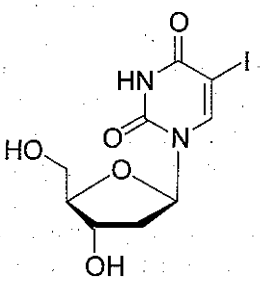
Inject reference solution(d). The relative retention times are about 0.55 for butylparaben, 0.7 for pseudoephedrine and 1.0 for ibuprofen. the resolution between the butylparaben peak and the pseudoephedrine peak and between the pseudoephedrine peak and the ibuprofen peak is not less than 2.0. The test is not valid unless the tailing factor for each analyte peak is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution(d) and the test solution.

Calculate the contents of $C_{13}H_{18}O_2$ and $C_{10}H_{15}NO,HCl$ in the tablets.

Storage. Store protected from moisture.

Idoxuridine



$C_9H_{11}IN_2O_5$ Mol. Wt. 354.1

Idoxuridine is 2'-deoxy-5-iodouridine.

Idoxuridine contains not less than 98.0 per cent and not more than 101.0 per cent of $C_9H_{11}IN_2O_5$, calculated on the dried basis.

Category. Antiviral (for topical use).

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *idoxuridine IPRS* or with the reference spectrum of idoxuridine. Examine the substances as dispersions containing 1 mg in 0.3 g of *potassium bromide IR*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum only at about 279 nm; absorbance at about 279 nm, about 0.65.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. Heat about 5 mg in a test-tube over a naked flame; a violet vapour is evolved.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *1 M sodium hydroxide* is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 5.5 to 6.5, determined in a 0.1 per cent w/v solution.

Specific optical rotation (2.4.22). +28.0° to +32.0°, determined in a 1.0 per cent w/v solution in *1 M sodium hydroxide*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *2-propanol*, 40 volumes of *chloroform* and 10 volumes of *strong ammonia solution*.

Test solution (a). Dissolve 0.4 g of the substance under examination in 10 ml of a mixture of 5 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the same solvent mixture.

Reference solution (a). Dilute 5.0 ml of test solution (b) to 100.0 ml with the same solvent mixture.

Reference solution (b). A solution containing 0.02 per cent w/v, each of, *5-iodouracil IPRS* and *2-deoxyuridine IPRS* in the same solvent mixture.

Reference solution (c). A solution containing 0.4 per cent w/v of *idoxuridine IPRS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air and repeat the development. After removal of the plate following the second development, dry it in a current of cold air and examine under ultraviolet light at 254 nm. The spots due to 5-iodouracil and 2'-deoxyuridine in the chromatogram obtained with reference solution (b) are more intense than any corresponding spots in the chromatogram obtained with test solution (a). Any other secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Iodide. Not more than 0.1 per cent, determined by the following method. Dissolve 0.25 g in 25 ml of *0.1 M sodium hydroxide*, 5 ml of *dilute hydrochloric acid* and sufficient *water* to produce 50 ml, allow to stand for 10 minutes and filter. To 25 ml of the filtrate add 5 ml of *hydrogen peroxide solution (10 vol)* and 10 ml of *chloroform* and shake. Any pink colour produced in the organic layer is not more intense than that obtained by repeating the procedure using 1 ml of a 0.033 per cent w/v solution of *potassium iodide* in place of the substance under examination.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa.

Assay. Weigh 0.3 g, dissolve in 50 ml of *dimethylformamide* and titrate with *0.1 M tetrabutylammonium hydroxide*,

determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03541 g of $C_9H_{11}IN_2O_5$.

Storage. Store protected from light.

Idoxuridine Eye Drops

Idoxuridine Eye Drops are a sterile solution of Idoxuridine in Purified Water.

Idoxuridine Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of idoxuridine, $C_9H_{11}IN_2O_5$.

Usual strength. 0.1 per cent w/v.

Identification

A. Dilute a suitable volume with *0.01 M sodium hydroxide* to produce a solution containing 0.003 per cent w/v of Idoxuridine. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 279 nm.

B. In the Assay, the chromatogram obtained with the reference solution (a) shows a peak that corresponds to the peak due to idoxuridine in the chromatogram obtained with the test solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the eye drops with sufficient of a solution of *sulphanilamide* (internal standard) to obtain a solution containing 0.08 per cent w/v of Idoxuridine and 0.0001 per cent w/v of the internal standard.

Reference solution. A solution containing 0.0004 per cent w/v each of *2'-deoxyuridine* and *5-bromo-2'-deoxyuridine*, 0.0008 per cent w/v of *5-iodouracil* and 0.0001 per cent w/v of *sulphanilamide* (internal standard).

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with endcapped octadecylsilane bonded to porous silica (10 µm) (Such as µBondapak C18),
- mobile phase: a mixture of 4 volumes of *methanol* and 96 volumes of *water*,
- flow rate: 1.7 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The order of elution of the peaks following the internal standard is deoxyuridine, iodouracil, bromodeoxyuridine and idoxuridine. Several peaks due to excipients may appear in the chromatogram obtained with the test solution before the peak due to the internal standard.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the ratio of the area of any peak due to 2'-deoxyuridine to the area of the peak due to sulphanilamide is not more than the ratio of the areas of the corresponding peaks in the chromatogram obtained with the reference solution (0.5 per cent). The ratio of the area of any peak due to 5-iodouracil to the area of the peak due to sulphanilamide is not more than the ratio of the areas of the corresponding peaks in the chromatogram obtained with the reference solution (1.0 per cent). The ratio of the area of any peak due to 5-bromo-2'-deoxyuridine to the area of the peak due to sulphanilamide is not more than the ratio of the areas of the corresponding peaks in the chromatogram obtained with the reference solution (0.5 per cent).

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Add 2 ml of a 10 per cent v/v solution of *ethanol (95 per cent)* to 15.0 ml of a solution prepared by diluting a measured volume of the eye drops with *water* if necessary to give a final concentration of 0.1 per cent w/v of Idoxuridine (solution A) and dilute to 20.0 ml with *water*.

Reference solution (a). Shake 0.1 g of *idoxuridine IPRS* with 50 ml of *water* until dissolved and then dilute to 100.0 ml with *water*. To 15.0 ml of the solution add 2.0 ml of a solution prepared by diluting 10 ml of a 1.2 per cent w/v of *sulphathiazole* (internal standard) in *ethanol (95 per cent)* to 100 ml with *water* (solution B), and dilute to 20.0 ml with *water*.

Reference solution (b). Add 2.0 ml of solution B to 15.0 ml of solution A and dilute to 20.0 ml with *water*.

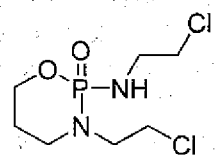
Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 87 volumes of *water* and 13 volumes of *methanol*,
- flow rate: 1.7 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Calculate the content of $C_9H_{11}IN_2O_5$ in the eye drops.

Storage. Store at a temperature not exceeding 30°. The eye drops should not be allowed to freeze.

Labelling. The label states that the eye drops should not be used for continuous periods of treatment exceeding 21 days.

Ifosfamide



$C_7H_{15}Cl_2N_2O_2P$ Mol. Wt. 261.1

Ifosfamide is (*RS*)-*N*,3-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide.

Ifosfamide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_{15}Cl_2N_2O_2P$, calculated on the anhydrous basis.

Category. Cytotoxic alkylating agent

Description. A white or almost white, fine, crystalline powder, hygroscopic.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ifosfamide IPRS* or with the reference spectrum of ifosfamide.

Tests

Solution A. Dissolve 5.0 g in *carbon dioxide-free water* and dilute to 50.0 ml with the same solvent.

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

Acidity or alkalinity. Dilute 5 ml of solution A to 50 ml with *carbon dioxide-free water*. To 10 ml of the solution, add 0.1 ml of *methyl red solution*. Not more than 0.1 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to red. To another 10 ml of the solution, add 0.1 ml of *phenolphthalein solution*. Not more than 0.3 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to pink.

Optical rotation (2.4.22). – 0.10° to + 0.10°, determined on solution A.

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

Solvent mixture. Equal volumes of *methanol* and *water*.

Mobile phase. A mixture of 10 volumes of *water*, 15 volumes of *methanol*, 25 volumes of *anhydrous acetic acid* and 50 volumes of *dichloromethane*.

Test solution. Dissolve 1.0 g of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.025 per cent, w/v each, of *ifosfamide impurity A IPRS (3-[(2-chloroethyl) amino]propyl dihydrogen phosphate IPRS)* and ifosfamide impurity C (*chloroethylamine hydrochloride)* in the solvent mixture.

Reference solution (b). A 0.015 per cent w/v solution of *ifosfamide impurity B IPRS (bis[3-[(2-chloroethyl) amino]propyl] dihydrogen diphosphate IPRS)* in the solvent mixture.

Reference solution (c). A solution containing 0.005 per cent w/v of *ethanolamine* (ifosfamide impurity D), 0.02 per cent w/v of *ifosfamide impurity A IPRS* and 0.08 per cent w/v of ifosfamide impurity C in the solvent mixture.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 115° for 45 minutes. At the bottom of a chromatographic tank, place an evaporating dish containing a 0.32 per cent w/v solution of *potassium permanganate* and add an equal volume of *dilute hydrochloric acid*, close the tank and allow to stand for 10 minutes. Place the plate whilst still hot in the tank, avoiding contact of the stationary phase with the solution, and close the tank. Leave the plate in contact with the chlorine vapour for 20 minutes. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed (about 20 minutes) and an area of coating below the points of application does not give a blue colour with a drop of *potassium iodide* and *starch solution*. Avoid prolonged exposure to cold air. Immerse the plate in a 0.1 per cent w/v solution of *tetramethylbenzidine* in *ethanol (95 per cent)* for 5 seconds. Allow the plate to dry and examine. In the chromatogram obtained with the test solution, any spot corresponding to ifosfamide impurity A or impurity C is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.25 per cent), any spot corresponding to impurity B is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.15 per cent); any other spot is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.15 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 3 clearly separated spots.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

Solvent mixture. Equal volumes of *methanol* and *dichloromethane*.

Mobile phase. 1 volume of *dichloromethane* and 10 volumes of *acetone*.

Test solution. Dissolve 0.2 g of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.005 per cent w/v each of *ifosfamide impurity E IPRS (3-chloro-N-(2-chloroethyl)propan-1-amine)* and *ifosfamide impurity F IPRS ((RS)-2-chloro-3-(2-chloroethyl)-1,3,2-oxazaphosphinane 2-oxide)* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *ifosfamide impurity E IPRS* and *ifosfamide IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 115° for 45 minutes. Proceed as described under Related substances test A. Any spot corresponding to ifosfamide impurity E or impurity F in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Chlorides (2.3.12). 25 ml of freshly prepared solution A complies with the limit test for chlorides (100 ppm).

Heavy metals (2.3.13). 12 ml of solution A complies with limit for heavy metals, method D (10 ppm), using 10 ml of *lead standard solution (1 ppm Pb)*.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (*2.2.29*).

NOTE — Use the solutions within 24 hours.

Solution A. Dissolve 50 mg of *ethyl parahydroxybenzoate* in 25 ml of *ethanol (95 per cent)* and dilute to 100.0 ml with *water*.

Test solution. To 0.15 g of the substance under examination, add 10.0 ml of solution A and dilute to 250.0 ml with *water*.

Reference solution. To 15 mg of *ifosfamide IPRS*, add 1.0 ml of solution A and dilute to 25.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 195 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to ifosfamide and ethyl parahydroxybenzoate is not less than 6.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_7H_{15}Cl_2N_2O_2P$.

Storage. Store protected from moisture.

Ifosfamide Injection

Ifosfamide Injection is a sterile solution of Ifosfamide in Water for Injections or a suitable liquid. It is prepared by dissolving Ifosfamide for Injection in the requisite amount of a suitable liquid immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

Storage. Ifosfamide Injection should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

Ifosfamide for Injection

Ifosfamide for Injection is a sterile material consisting of Ifosfamide with or without excipients. It is supplied in a sealed container.

CAUTION — Ifosfamide is Cytotoxic. Carry out the procedures described below exercising appropriate precautions.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Ifosfamide Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ifosfamide, $C_7H_{15}Cl_2N_2O_2P$.

Usual strength. 1000 mg per ml.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ifosfamide IPRS* or with the reference spectrum of ifosfamide.

Tests

Solution A. Dissolve 5.0 g in *carbon dioxide-free water* and dilute to 50.0 ml with the same solvent.

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than YS7 (2.4.1).

pH (2.4.24). 4.0 to 7.0, determined in 8.0 per cent w/v solution.

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. Equal volumes of *methanol* and *water*.

Mobile phase. A mixture of 10 volumes of *water*, 15 volumes of *methanol*, 25 volumes of *anhydrous acetic acid* and 50 volumes of *dichlormethane*.

Test solution. Dissolve a quantity of the sealed container in the solvent mixture to obtain a solution containing 10 per cent w/v of Ifosfamide.

Reference solution (a). A solution containing 0.025 per cent w/v each of *ifosfamide impurity A IPRS (3-[(2-chloroethyl) amino]propyl dihydrogen phosphate IPRS)* and *chloroethylamine hydrochloride* (ifosfamide impurity C) in the solvent mixture.

Reference solution (b). A 0.015 per cent w/v solution of *ifosfamide impurity B IPRS (bis[3-[(2-chloroethyl)amino] propyl] dihydrogen diphosphate IPRS)* in the solvent mixture.

Reference solution (c). A solution containing 0.005 per cent w/v of *ethanolamine* (ifosfamide impurity D), 0.02 per cent w/v of *ifosfamide impurity A IPRS* and 0.08 per cent w/v of *chloroethylamine hydrochloride* (ifosfamide impurity C) in the solvent mixture.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 115° for 45 minutes. At the bottom of a tank, place an evaporating dish containing a 0.32 per cent w/v solution of *potassium permanganate* and add an equal volume of *dilute hydrochloric acid*, close the tank and allow to stand for 10 minutes. Place the plate whilst still hot in the tank, avoiding contact of the stationary phase with the solution, and close the tank. Leave the plate in contact with the chlorine vapour for 20 minutes. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed (about 20 minutes) and an area of coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution*. Avoid prolonged exposure to cold air. Immerse the plate in a 0.1 per cent w/v solution of *tetramethylbenzidine* in *ethanol (95 per cent)* for 5 seconds. Allow the plate to dry and examine. In the chromatogram obtained with the test solution, any spot corresponding to ifosfamide impurity A or impurity C is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.25 per cent), any spot corresponding to ifosfamide impurity B is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b) (0.15 per cent). Any other spot is not more intense than the principal spot in the chromatogram obtained with reference solution (b) (0.15 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows three clearly separated spots.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. Equal volumes of *methanol* and *water*.

Mobile phase. 1 volume of *dichloromethane* and 10 volumes of *acetone*.

Test solution. Dissolve a quantity of the contents of the sealed container in the solvent mixture to obtain a solution containing 2.0 per cent w/v of Ifosfamide.

Reference solution (a). A solution containing 0.005 per cent w/v, each of, *ifosfamide impurity E IPRS* and *ifosfamide impurity F IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *ifosfamide impurity E IPRS* and *ifosfamide impurity F IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 115° for 45 minutes. At the bottom of a tank, place an evaporating dish containing a 0.32 per cent w/v solution of *potassium permanganate* and add an equal volume of *dilute hydrochloric acid*, close the tank and allow to stand for 10 minutes. Place the plate whilst still hot in the tank, avoiding contact of the stationary phase with the solution, and close the tank. Leave the plate in contact with the chlorine vapour for 20 minutes. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed (about 20 minutes) and an area of coating below the points of application does not give a blue colour with a drop of *potassium iodide and starch solution*. Avoid prolonged exposure to cold air. Immerse the plate in a 0.1 per cent w/v solution of *tetramethylbenzidine* in *ethanol (95 per cent)* for 5 seconds. Allow the plate to dry and examine. In the chromatogram obtained with the test solution, any spot corresponding to ifosfamide impurity E or impurity F is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.25 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Water (2.3.43). Not more than 0.5 per cent w/w, determined on 1.0 g.

Bacterial endotoxins (2.2.3). Not more than 0.125 Endotoxin Unit per mg of ifosfamide.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of contents of the sealed container in the mobile phase to obtain a solution containing 0.06 per cent w/v of Ifosfamide.

Reference solution. A 0.06 per cent w/v solution of *ifosfamide IPRS* in the mobile phase.

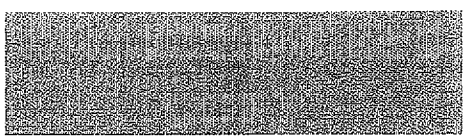
Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Zorbax SB-C18),
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 195 nm,
- injection volume: 20 µl.

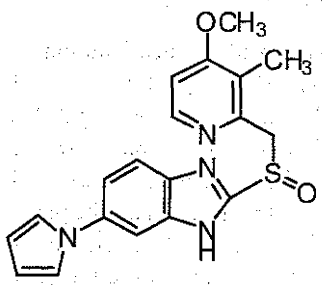
Inject the reference solution and the test solution.

Calculate the content of $C_7H_{15}Cl_2N_2O_2P$ in the injection.

Storage. Store protected from light.



Ilaprazole



$C_{19}H_{18}N_4O_2S$ Mol Wt. 366.4

Ilaprazole is 2-[(4-Methoxy-3-methylpyridin-2-yl) methylsulfinyl]-6-pyrrol-1-yl-1*H*-benzimidazole.

Ilaprazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{18}N_4O_2S$, calculated on the dried basis.

Category. Antacid.

Description. A white to brown powder.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in *acetonitrile* and dilute to 25.0 ml with *acetonitrile*.

Reference solution. A 0.1 per cent w/v solution of *ilaprazole IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) .
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 1.42 g of *disodium hydrogen orthophosphate* in 1000 ml of *water* adjusted to pH 5.5 and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. In the chromatogram obtained with test solution the area of any secondary peak is not more than 0.5 per cent and the area of sum of the areas of all the secondary peaks is not more than 1.5 per cent, calculated by area normalization.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° for 3 hours under vacuum.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *acetonitrile* and dilute to 50.0 ml with *acetonitrile*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *ilaprazole IPRS* in *acetonitrile*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

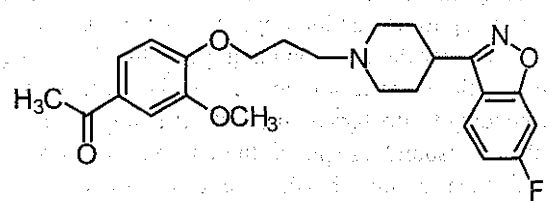
Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and test solution.

Calculate the content of $C_{19}H_{18}N_4O_2S$.

Iloperidone



$C_{24}H_{27}FN_2O_4$ Mol Wt. 426.5

Iloperidone is 1-(4-{3-{4-(6-Fluoro-1,2-benzoxazol-3-yl)piperidino]-propoxy}-3-methoxyphenyl)ethanone.

Iloperidone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{24}H_{27}FN_2O_4$, calculated on the dried basis.

Category. Antipsychotic

Description. A white to off white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *iloperidone IPRS* or with the reference spectrum of iloperidone.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of 0.1 per cent v/v of *orthophosphoric acid* in *water* and *acetonitrile.*

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *iloperidone IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 20°,
- mobile phase: A. *0.02 M sodium perchlorate*, adjusted to pH 3.0 with *perchloric acid,*
  B. a mixture of 80 volumes of *acetonitrile* and 20 volumes of *methanol,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
5	65	35
18	48	52
28	48	52
35	30	70
40	30	70
40.5	65	35
48	65	35

Inject the reference solution .The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° for 3 hours.

Assay. Dissolve 0.4 g in 50.0 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04265g of $C_{24}H_{27}FN_2O_4$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Iloperidone Tablets

Iloperidone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of $C_{24}H_{27}FN_2O_4$.

Usual strengths. 1 mg; 2 mg; 4 mg; 6 mg; 8 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium.

Determine by liquid chromatography (2.4.14).

Use solvent mixture as given under Related substances.

Test solution. Centrifuge the medium at 3000 rpm for 10 minutes. Dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *iloperidone IPRS* in the solvent mixture with the aid of ultrasound and futher dilute with dissolution medium to obtain a solution of the same concentration as that of the test solution.

Use chromatographic system as described under Assay using injection volume 100 µl.

Inject the reference solution and the test solution.

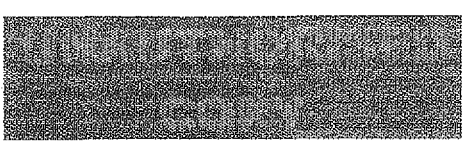
Calculate the content of $C_{24}H_{27}FN_2O_4$ in the medium.

Q. Not less than 70.0 per cent of the stated amount of $C_{24}H_{27}FN_2O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of buffer solution prepared by dissolving 3.4g of *potassium dihydrogen phosphate* in 1000 ml of *water*, add 1.0 ml of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid* and 50 volumes of *acetonitrile.*

Test solution. Disperse a quantity of powdered tablets containing 10 mg of Iloperidone in 15 ml of the solvent mixture



with the aid of ultrasound for 45 minutes and dilute to 25.0 ml with the solvent mixture, filter.

Reference solution. A 0.0002 per cent w/v solution of *iloperidone IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 64 volumes of buffer solution prepared by dissolving 3.4 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, add 1.0 ml of *triethylamine*, adjusted to pH 5.0 with *orthophosphoric acid*, 21 volumes of *acetonitrile* and 15 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 229 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay with the following modification.

Test solution. Disperse 1 intact tablet in the solvent mixture with the aid of ultrasound for 45 minutes and dilute to obtain a solution containing 0.008 per cent w/v solution of Iloperidone with the solvent mixture, filter.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{27}FN_2O_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of buffer solution prepared by dissolving 3.4 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, add 1.0 ml of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid* and 50 volumes of *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 8 mg of Iloperidone in 70 ml of the solvent mixture with the aid of ultrasound for 45 minutes with intermittent shaking and dilute to 100.0 ml with the solvent mixture, filter.

Reference solution. A 0.008 per cent w/v solution of *iloperidone IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature 45°,
- mobile phase: a mixture of 64 volumes of buffer solution prepared by dissolving 3.4 g of *potassium dihydrogen phosphate* in 1000 ml in *water*, add 1.0 ml of *triethylamine*, adjusted to pH 5.0 with *orthophosphoric acid*, 21 volumes of *acetonitrile* and 15 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 229 nm,
- injection volume: 20 µl.

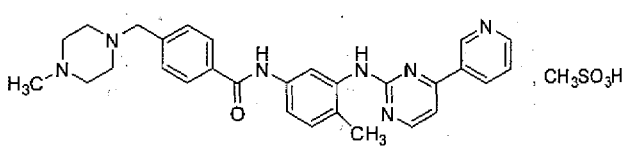
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{27}FN_2O_4$ in the tablets.

Storage. Store protected from light and moisture.

Imatinib Mesylate



$C_{30}H_{35}N_7SO_4$ Mol. Wt. 589.7

Imatinib Mesylate is 4-[(4-Methylpiperazin-1-yl)methyl]-*N*-[4-methyl-3-[[4-(pyridine.-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide methanesulfonate.

Imatinib Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{30}H_{35}N_7SO_4$, calculated on the anhydrous basis.

Category. Cytotoxic

Description. A white or almost white, slightly brownish or yellowish powder, yellow or pale yellow, very hygroscopic, for the amorphous form. It shows polymorphism (2.5.11).

Identification

Determine by infrared adsorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *imatinib mesylate IPRS* or with the reference spectrum of imatinib mesylate.

Tests

Impurity F. Determine by liquid chromatography (2.4.14) coupled with mass spectrometry.

Solvent mixture. 30 volumes of *acetonitrile* and 70 volumes of *water.*

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.0001 per cent w/v solution of *imatinib impurity F IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 3.0mm, end-capped packed with octadecylsilane bonded to amorphous organosilica (3.5 µm),
- column temperature: 40°,
- mobile phase: A. a buffer solution prepared by dissolving 1.26 g of *ammonium formate* in 1000 ml of *water,* adjusted to pH 3.4 with *anhydrous formic acid,*
  B. a 0.05 per cent v/v solution of *anhydrous formic acid* in *acetonitrile,*
- flow rate: 0.5 ml per minute,
- detection. mass detector.

The following settings have been found to be suitable and are given as examples; if the detector has different setting parameters, adjust the detector settings so as to comply with system suitability criterion,

- Ionisation. ESI-positive,
- Detection m/z (SIM): 278.2,
- Gas temperature: 350°,
- Drying gas flow: 12 litre per minute,
- Nebuliser pressure: 414 kpa;
- Capillary voltage (V cap): 3 Kv,
- Injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
6	80	0
10	20	80
15	20	80
15.1	80	20
20	80	20

NOTE — MS acquisition can be started at 3.5 minutes and stopped at 6 minutes,during non acquisition the eluent is directed to waste.

Inject the reference solution. The test is not valid unless the signal-to-noise ratio of the principal peak is not less than 20 and the relative standard deviation for replicate injections is not more than 10 per cent.

Inject the reference solution and the test solution. The area of any peak corresponding to imatinib impurity F is not more than the area of the principal peak in a chromatogram obtained with the reference solution (0.002 per cent).

Impurity H. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of *acetonitrile* and 70 volumes of *water.*

Test solution. Dissolve 75mg of the substance under examination in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Reference solution (a). Dissolve the contents of a vial of *imatinib impurity A IPRS* in 1.0 ml of the solvent mixture.

Reference solution (b). A 0.003 per cent w/v solution of *imatinib impurity H IPRS* in the solvent mixture.

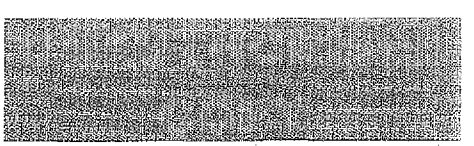
Reference solution (c). Dilute 5.0 ml of reference solution (b) to 50.0 ml with the solvent mixture.

Reference solution (d). Dissolve 0.15 g of the substance under examination in the solvent mixture, add 1.0 ml of each, reference solution (a) and (b) and dilute to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, endcapped packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: A. a mixture of 70 volumes of a buffer solution prepared by dissolving 2.3 g of *sodium octane sulphonate monohydrate* in 700 ml of *water,* 30 volumes of *acetonitrile* and 0.12 volume of *dilute orthophosphoric acid,*
  B. a mixture of 10 volumes of a buffer solution, prepared by dissolving 2.3 *g sodium octane sulphonate monohydrate* in 100 ml of *water,* 90 volumes of *acetonitrile* and add 0.12 volume of *dilute orthophosphoric acid,*
  a gradient programme using the condition given below,
- flow rate: 2.3 ml per minute,
- spectrophotometer set at 227nm,
- injection volume:10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
6	98	2
8	20	80
10	20	80
10.1	98	2
15	98	2



Name	Relative retention time
Imatinib impurity A[1]	0.17
Imatinib impurity H[2]	0.2
Imatinib (Retention time: about 8 minutes)	1.0

[1](2*E*)-3-(dimethylamino)-1-(pyridine-3-yl)prop-2-en-1-one,

[2]1-(pyridine-3-yl)ethan-1-one.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to imatinib impurity A and H is not less than 1.5.

Inject reference solution (c) and the test solution. The area of any peak corresponding to imatinib impurity H is not more than the area of principal peak in a chromatogram obtained with reference solution (c) (0.02 per cent).

Related substances. Determine by liquid chromatography (2.4.14), as described under Impurity H with the following modifications.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dissolve 1 mg of *imatinib system suitability IPRS* (containing impurities A, B, C, D and J) in the solvent mixture and dilute to 2 ml with the solvent mixture.

Reference solution (c). A 0.05 per cent w/v solution of *imatinib mesylate IPRS* in the solvent mixture.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
16	98	2
30	50	50
30.1	98	2
35	98	2

Name	Relative retention time	Correction factor
Imatinib impurity A[1]	0.2	2.2
Imatinib impurity B[2]	0.6	2.0
Imatinib impurity J[3]	0.9	—
Imatinib (Retention time: about 11 minutes)	1.0	—
Imatinib impurity C[4]	1.2	—
Imatinib impurity D[5]	2.3	—

[1](2E)-3-(dimethylamino)-1-(pyridine-3-yl)prop-2-en-1-one,

[2]*N*-(3-carbamimidamido-4-methylphenyl)-4-[(4-methylpiperazin-1-yl)methyl]benzamide,

[3]4-[(4-methyl-4-oxidopiperazin-1-yl)methyl]-*N*-[4-methyl-3-[[4-(pyridine-3-yl)pyrimidin-2-yl]amino]phenyl] benzamide,

[4]*N*-[4-methyl-3-[[4-(pyridine-3-yl)pyrimidin-2-yl]amino]phenyl]-4-(piperazin-1-ylmethyl)benzamide(desmethylimatinib),

[5]1-methyl-1,4-bis[4-[[4-methyl-3-[[4-(pyridine-3-yl)pyrimidin-2-yl]amino]phenyl]carbamoyl]benzyl]piperazin-1-ium(imatinib dimer).

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to imatinib and imatinib impurity C is not less than 3.0, the peak-to-valley ratio between the peaks due to imatinib impurity J and imatinib is not less than 1.3. Where *Hp* is height above the baseline of the peak due to imatinib impurity J and *Hv* is height above the baseline of the lowest point of the curve separating this peak from the peak due to imatinib in the chromatogram obtained with reference solution (b) and signal-to-noise ratio for the principal peak is not less than 45 in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to imatinib impurity C is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any peak corresponding to imatinib impurity D is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any peak corresponding to imatinib impurity A and B, each of, is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution(a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.12).1.0 gm complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18).Not more than 0.1 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{30}H_{35}N_7SO_4$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Imatinib Capsules

Imatinib Mesylate Capsules

Imatinib Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of stated amount of imatinib, $C_{29}H_{31}N_7O$.

Usual strength. 100 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of mixed contents of 20 capsules containing about 20 mg of imatinib with 100.0 ml of the mobile phase.

Reference solution. A 0.024 per cent w/v solution of *imatinib mesylate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of 1 per cent *ammonium acetate* and 60 volumes of *acetonitrile,*
- flow rate: 0.7 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{31}N_7O$ in the capsules.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of equivalent amount of Imatinib.

Imatinib Tablets

Imatinib Mesylate Tablets

Imatinib Mesylate Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of imatinib, $C_{29}H_{31}N_7O$.

Usual strengths. 100 mg; 400 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle) with sinkers,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.011 per cent w/v solution of *imatinib mesylate IPRS* prepared by dissolving in minimum amount of *water* and diluting with the dissolution medium.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 65 volumes of buffer solution prepared by dissolving 3.12 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 ml of *water*, adding 1 ml of *triethylamine*, adjusted to pH 3.5 with *orthophosphoric acid* and 35 volumes of a mixture of 50 volumes of *methanol* and 50 volumes of *acetonitrile,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{31}N_7O$ in the medium.

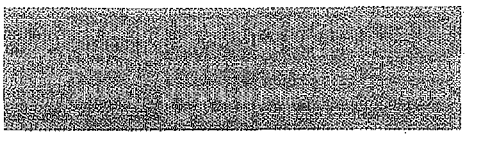
Q. Not less than 75 per cent of the stated amount of $C_{29}H_{31}N_7O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 125 mg of imatinib in 70 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 250.0 ml with the mobile phase.

Reference solution. A 0.0006 per cent w/v solution of *imatinib mesylate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,



- mobile phase: a mixture of 75 volumes of buffer solution prepared by dissolving 1.0 g of *ammonium acetate* in 1000 ml *water* and 25 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogarm obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 125 mg of imatinib in 100 ml of *methanol* with the aid of ultrasound for 20 minutes and dilute to 250.0 ml with *methanol*, centrifuge. Dilute 10.0 ml of the solution to 100.0 ml with *water*.

Reference solution. A 0.006 per cent w/v solution of *imatinib mesylate IPRS* prepared by dissolving in *methanol* and diluted with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 55 volumes of buffer solution prepared by dissolving 3.12 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 ml *water*, adding 1 ml of *triethylamine*, adjusted to pH 3.5 with *orthophosphoric acid* and 45 volumes of a mixture of equal volumes of *methanol* and *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

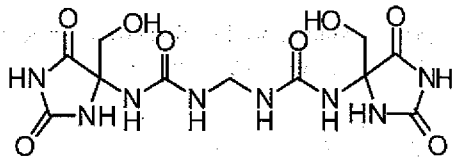
Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{31}N_7O$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of Imatinib.

Imidurea



$C_{11}H_{16}N_8O_8$ Mol. Wt. 388.3

Imidurea is Methylenebis[3-(4-hydroxymethyl-2,5-dioxoimidazolidin-4-yl)urea].

Imidurea contains not less than 26.0 per cent and not more than 28.0 per cent of nitrogen, N, calculated on the dried basis.

Description. A white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *imidurea IPRS* or with the reference spectrum of imidurea.

Tests

Appearance of solution. Dissolve 3.0 g in 7.0 ml of *water* in a test tube; the solution is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 6.0 to 7.5, determined in a 1.0 per cent w/v solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

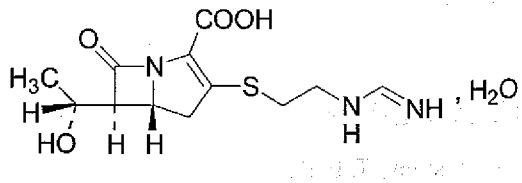
Sulphated ash (2.3.18). Not more than 3.0 per cent.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1 g by drying in vacuum over *phosphorus pentoxide* for 48 hours.

Nitrogen (2.3.30). Determined on 150 mg, using method C.

Storage. Store protected from moisture.

Imipenem



$C_{12}H_{17}N_3O_4S,H_2O$ Mol. Wt. 317.4

Imipenem is (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-3-[[2-[(iminomethyl)amino]ethyl]sulphanyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate.

Imipenem contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{12}H_{17}N_3O_4S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white to almost white or pale yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *imipenem monohydrate IPRS* or with the reference spectrum of imipenem monohydrate.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *phosphate buffer pH 7.0* is not more opalescent than opalescence standard OS2 (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 4.5 to 7.0 determined in 0.5 per cent w/v solution in *water*.

Specific optical rotation (2.4.22). + 84.0° to + 89.0°, determined in a 0.5 per cent w/v solution in *phosphate buffer pH 7.0*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Keep the solutions in an ice-bath and use within 8 hours of preparation.

Solvent mixture. 0.7 volume of *acetonitrile* and 99.3 volumes of a 0.0135 per cent w/v solution of *dipotassium hydrogen phosphate,* adjusted to pH 6.8 with *orthophosphoric acid.*

Test solution. Dissolve 40 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). A 0.04 per cent w/v solution of *imipenem IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (c). Heat 20 ml of the test solution, previously adjusted to pH 10.0 with *sodium hydroxide solution* at 80° for 5 minutes (in situ preparation of impurity A).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.7 volumes of *acetonitrile* and 99.3 volumes of a 0.87 per cent w/v solution of *dipotassium hydrogen phosphate,* adjusted to pH 7.3 with *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (c). The relative retention time with reference to imipenem for imipenem impurity A is about 0.8 and the resolution between the peaks due to imipenem impurity A and imipenem is not less than 3.5.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to imipenem impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any other secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent) and the sum of the areas of all the secondary peaks, other than the peak corresponding to imipenem impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on 1.0 g.

Water (2.3.43). 5.0 per cent to 8.0 per cent, determined on 0.1 g. Use an iodosulfurous reagent containing imidazole instead of pyridine and a clean container for each determination.

Assay. Determine by liquid chromatography (2.4.14).

NOTE —Keep the solutions in an ice-bath and use within 8 hours of preparation.

Test solution. Dissolve about 40 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.04 per cent w/v solution of *imipenem monohydrate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as Bondapak C18),
- mobile phase: a solution prepared by dissolving 54 mg of *monobasic potassium phosphate* in 360 ml of *water*, adjusted to pH 6.8 with *0.5 M sodium hydroxide or 0.5 M orthophosphoric acid*, dilute to 400 ml with *water* and filter,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 600 theoretical plates, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{12}H_{17}N_3O_4S$.

Imipenem intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial Endotoxins (2.2.3). Not more than 0.17 Endotoxin Unit per mg of imipenem.

Imipenem intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store in an airtight container in a refrigerator (2° to 8°).

Labelling. The label states, where applicable, that the substance is sterile and is free from bacterial endotoxins.

Imipenem and Cilastatin Injection

Imipenem and Cilastatin Injection is a sterile mixture of Imipenem, Cilastatin Sodium, and Sodium Bicarbonate.

The injection is constituted as per the labelling requirements.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Imipenem and Cilastatin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amounts of imipenem, $C_{12}H_{17}N_3O_4S$ and cilastatin, $C_{16}H_{26}N_2O_5S$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.5 to 8.5, when constituted as directed on the label.

Bacterial endotoxins (2.2.3). Not more than 0.17 Endotoxin Unit per mg of imipenem and not more than 0.17 Endotoxin Unit per mg of cilastatin.

Loss on drying (2.4.19). Not more than 3.5 per cent, determined on 0.1 g, by drying in an oven at 60° for 3 hours at a pressure not exceeding 0.7 kPa.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the following solutions immediately before use.

Buffer solution pH 6.8. Dissolve 0.14 g of *monobasic potassium phosphate* in 900 ml of *water*, adjusted to pH 6.8 with *0.5M sodium hydroxide* or *0.5 M orthophosphoric acid* and dilute to 1000 ml with *water* and filter.

Test solution. Reconstitute the Imipenem and Cilastatin Injection and dilute to 100.0 ml with buffer solution pH 6.8 and mix. Dilute an accurately measured volume of the solution with buffer solution pH 6.8 to obtain a solution having a concentration of 0.05 per cent of imipenem.

Reference solution. A solution containing 0.05 per cent w/v, each of, *imipenem monohydrate IPRS* and *cilastatin ammonium IPRS* in buffer solution pH 6.8.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: dissolve 2.0 g of *sodium 1-hexane-sulphonate* in 800 ml of buffer solution pH 6.8, adjusted to pH 6.8 with *0.5 M sodium hydroxide* or *0.5 M orthophosphoric acid*, dilute to 1000 ml with buffer solution pH 6.8 and filter,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 600 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

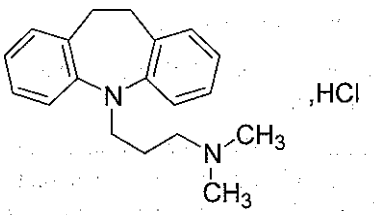
Inject the reference solution and the test solution.

Calculate the contents of $C_{12}H_{17}N_3O_4S$ and $C_{16}H_{26}N_2O_5S$ in the injection.

Storage. Store protected from moisture, in a single dose or multiple dose container.

Labelling. The label states that the constituted solution should be solubilized in a suitable parenteral fluid prior to intravenous infusion.

Imipramine Hydrochloride



$C_{19}H_{24}N_2,HCl$ Mol. Wt. 316.9

Imipramine Hydrochloride is 10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine-5-(dimethylaminopropyl) hydrochloride.

Imipramine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{19}H_{24}N_2,HCl$, calculated on the dried basis.

Category. Antidepressant.

Description. A white or slightly yellow, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *imipramine hydrochloride IPRS* or with the reference spectrum of imipramine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum only at about 250 nm and a shoulder at about 270 nm; absorbance at about 250 nm, about 0.52.

C. Dissolve 5 mg in 2 ml of *nitric acid*; an intense blue colour is produced.

D. Dissolve 50 mg in 3 ml of *water* and add 1 drop of a 2.5 per cent w/v solution of *quinhydrone* in *methanol*; no red colour is produced within 15 minutes.

E. 20 mg gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. Triturate with a glass rod 3.0 g with 20 ml of *carbon dioxide-free water* and dilute to 30 ml with the same solvent (solution A). Solution A is clear (2.4.1). Immediately after preparation dilute the solution with an equal volume of *water*. The resulting solution is not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 3.6 to 5.0, determined in solution A immediately after preparation.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with end-capped polar-embedded octadecylsilane amorphous organosilica polymer (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of a 0.52 per cent w/v solution of *dipotassium hydrogen phosphate* previously adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

The relative retention time with reference to imipramine (retention time: about 7 minutes) for imipramine impurity B (depramine) is about 0.7.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. The area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.5 g, dissolve in 50 ml of *chloroform*, add 10 ml of *mercuric acetate solution* and titrate with *0.1 M perchloric acid*, using 0.5 ml of *metanil yellow solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03169 g of $C_{19}H_{24}N_2,HCl$.

Storage. Store protected from light.

Imipramine Tablets

Imipramine HydrochlorideTablets

Imipramine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of imipramine hydrochloride, $C_{19}H_{24}N_2$,HCl. The tablets are coated.

Usual strengths. 10 mg; 25 mg.

Identification

Triturate a quantity of the powdered tablets containing about 0.25 g of Imipramine Hydrochloride with 10 ml of *chloroform*, filter, evaporate the filtrate to low bulk, add *ether* until a turbidity is produced, and allow to stand. The precipitate, after recrystallisation from *acetone*, melts at about 172° (2.4.21), and complies with the following tests.

A. Dissolve 5 mg in 2 ml of *nitric acid;* an intense blue colour is produced.

B. Dissolve 50 mg in 3 ml of *water* and add 1 drop of a 2.5 per cent w/v solution of *quinhydrone* in *methanol*; no red colour is produced within 15 minutes.

C. 20 mg gives reaction (A) of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium: 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 100 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium, if necessary. Measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7). Calculate the content imipramine hydrochloride, $C_{19}H_{24}N_2$,HCl in the medium from the absorbance obtained from a solution of known concentration of *imipramine hydrochloride IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{19}H_{24}N_2$,HCl.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 55 volumes of *ethyl acetate*, 35 volumes of *glacial acetic acid*, 5 volumes of *hydrochloric acid* and 5 volumes of *water*.

NOTE — Prepare the following solutions immediately before use.

Test solution. Shake a quantity of the powdered tablets containing 0.2 g of Imipramine Hydrochloride with three quantities, each of 10 ml, of *chloroform*, filter the combined chloroform extracts, evaporate to dryness and dissolve the residue in 10 ml of *methanol*.

Reference solution (a). Dilute 3 volumes of the test solution to 1000 volumes with *methanol*.

Reference solution (b). A 0.006 per cent w/v solution of *iminodibenzyl IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, remove the plate, allow the solvent to evaporate for 5 minutes, spray with a 0.5 per cent w/v solution of *potassium dichromate* in *sulphuric acid (20 per cent)* and examine immediately. In the chromatogram obtained with the test solution any spot corresponding to iminodibenzyl is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

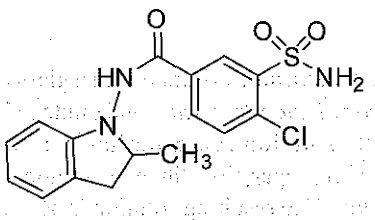
Uniformity of content. Complies with the test stated under Tablets.

Powder one tablet, shake with 25 ml of *0.1 M hydrochloric acid* for 30 minutes, add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate to 50.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{19}H_{24}N_2$,HCl in the tablet taking 264 as the specific absorbance at 250 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of Imipramine Hydrochloride, shake with 75 ml of *0.1 M hydrochloric acid* for 30 minutes, dilute to 100.0 ml with the same solvent and filter through a sintered-glass filter. Dilute 10.0 ml to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{19}H_{24}N_2$, HCl taking 264 as the specific absorbance at 250 nm.

Indapamide



$C_{16}H_{16}ClN_3O_3S$ Mol. Wt. 365.8

Indapamide is 4-chloro-*N*-(2-methyl-1-indolinyl)-3-sulphamoylbenzamide.

Indapamide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{16}H_{16}ClN_3O_3S$, calculated on the dried basis.

Category. Diuretic.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *indapamide IPRS* or with the reference spectrum of indapamide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light and prepare the solutions immediately before use or maintain them at 4 °.

Solvent mixture. Equal volumes of *acetonitrile* and *methanol.*

Test solution. Dissolve 20 mg of the substance under examination in 7 ml of the solvent mixture and dilute to 20.0 ml with a 0.02 per cent w/v solution of *sodium edetate.*

Reference solution (a). Dissolve 3 mg of *indapamide impurity B IPRS (4-chloro-N-(2-methyl-1H-indol-1-yl)-3-sulphamoyl benzamide IPRS)* in 3.5 ml of the solvent mixture and dilute to 10.0 ml with a 0.02 per cent w/v solution of *sodium edetate.* To 1.0 ml of the solution, add 35 ml of the solvent mixture and dilute to 100.0 ml with 0.02 per cent w/v solution of *sodium edetate.*

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with a mixture of 17.5 volumes of *acetonitrile*, 17.5 volumes of *methanol* and 65 volumes of a 0.02 per cent w/v solution of *sodium edetate.* Dilute 1.0 ml of the solution to 20.0 ml with a mixture of 17.5 volumes of *acetonitrile*, 17.5 volumes of *methanol* and 65 volumes of a 0.02 per cent w/v solution of *sodium edetate.*

Reference solution (c). Dissolve 25 mg of *indapamide IPRS* and 45 mg of *methylnitrosoindoline IPRS* in 17.5 ml of the solvent mixture and dilute to 50.0 ml with 0.02 per cent w/v solution of *sodium edetate.*

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 0.1 volume of *glacial acetic acid*, 17.5 volumes of *acetonitrile*, 17.5 volumes of *methanol*, 65 volumes of 0.02 per cent w/v solution of *sodium edetate*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

The retention time of indapamide peak is about 11 minutes.

Inject reference solution (b) and (c). The test is not valid unless the resolution between the peaks due to indapamide and indapamide impurity A is not less than 4.0 in the chromatogram obtained with reference solution (c) and signal-to-noise ratio is not less than 6.0 for the principal peak in the chromatogram obtained with reference solution (b).

Inject reference solution (a), (b) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to indapamide impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Impurity A. Determine by liquid chromatography (2.4.14).

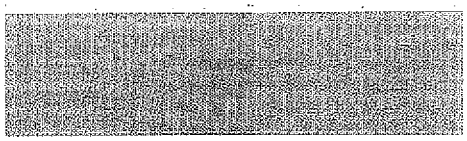
NOTE — Carry out the test protected from light.

Test solution. Dissolve 25 mg of the substance under examination in 1 ml of *acetonitrile* and dilute to 10.0 ml with *water.* Shake for 15 minutes and allow to stand at 4° for 1 hour and filter.

Reference solution. Dissolve 25 mg of the substance under examination in 1.0 ml of a 0.0000125 per cent w/v solution of *indapamide impurity A IPRS (methylnitrosoindoline IPRS)* in *acetonitrile* and dilute to 10.0 ml with *water.* Shake for 15 minutes and allow to stand at 4° for 1 hour and filter.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 7 volumes of *acetonitrile*, 20 volumes of *tetrahydrofuran* and 73 volumes of a 0.15 per cent w/v solution of *triethylamine*, adjusted to pH 2.8 with *orthophosphoric acid*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the signal-to-noise ratio is not less than 3.0 for the peak due to indapamide impurity A appearing just before the peak due to indapamide and the peak-to-valley-ratio is not less than 6.7, where H_p is the height above the baseline of the peak due to



indapamide impurity A and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to indapamide.

Inject the reference solution and the test solution. The area of the peak due to indapamide impurity A is not more than the difference between the areas of the peaks due to indapamide impurity A in the chromatograms obtained with the reference solution and the test solution (5 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. A 0.5 per cent w/v solution of *4-chloroacetanilide* in *methanol*.

Test solution. Dissolve about 0.1 g of the substance under examination in 5.0 ml of *internal standard solution* and dilute to 100.0 ml with the mobile phase.

Reference solution. A solution containing 0.1 per cent w/v of *indapamide IPRS* and 0.025 per cent w/v of *4'-chloroacetanilide* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *water*, 17.5 volumes of *acetonitrile*, 17.5 volumes of *methanol* and 0.1 volume of *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5 µl.

The relative retention time with reference to indapamide for *4'*- chloroacetanilide is about 0.65.

Inject the reference solution. The test is not valid unless the resolution between the principal peak and any adjacent peak is not less than 2.0, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent for the principal peak.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{16}ClN_3O_3S$.

Storage. Store protected from moisture.

Indapamide Prolonged-release Tablets

Indapamide Sustained-release Tablets; Indapamide Extended-release Tablets

Indapamide Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Indapamide Prolonged-release Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of indapamide, $C_{16}H_{16}ClN_3O_3S$.

Usual strengths. 1.5 mg; 2.5 mg.

Identification

A. Determine by thin layer chromatography (2.4.17), using the plate coated with *silica gel GF 254*.

Mobile phase. A mixture of 20 volumes of *acetone* and 80 volumes of *toluene*.

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Indapamide with 10 ml of *acetone*, mix for 15 minutes and filter.

Reference solution. A 0.5 per cent w/v solution of *indapamide IPRS* in *acetone*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to raise 12 cm. Dry the plate in air and examine under the ultraviolet light at 254 nm. Spray the plate with a solution prepared by mixing 10 volumes of *potassium iodobismuthate solution* and 20 volumes of *glacial acetic acid* and diluting to 100 volumes with *water* and examine again. Finally, spray the plate with a 5.0 per cent w/v solution of *sodium nitrite* in a mixture of equal volume of *water* and *ethanol (95 per cent)* and examine again.The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution. Complies with the tests stated under tablets.

Uniformity of content. Complies with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using following modification.

Test solution. Disperse one tablet in 20 ml of ethanol (95 per cent). Mix with the aid of ultrasound for 15 minutes. Cool and dilute to 25 ml with ethanol (95 per cent). Dilute as necessary to obtain a solution containing 0.005 per cent w/v of indapamide in ethanol (95 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 25 mg of indapamide in 70 ml of *ethanol (95 per*

cent) mix with the aid of ultrasound, dilute to 100.0 ml with *ethanol (95 per cent)*, mix and centrifuge. Dilute 5.0 ml of the supernatant liquid to 25.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of a 0.00025 per cent w/v solution of *indapamide impurity B IPRS* in *ethanol (95 per cent)* to 10.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of a 0.00025 per cent w/v solution of *4-chloro-3-sulphamoyl benzoic acid* in *ethanol (95 per cent)* to 10.0 ml with the mobile phase.

Reference solution (d). Mix 1.0 ml of the test solution, 1.0 ml of 0.00025 per cent w/v solution of *indapamide impurity B IPRS* in *ethanol (95 per cent)* and 1.0 ml of 0.00025 per cent w/v solution of *4-chloro-3-sulphamoyl benzoic acid* in *ethanol (95 per cent)* and dilute to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 6 volumes of a solution containing 5.0 per cent w/v solution of *sodium lauryl sulphate* and 3.0 per cent v/v solution of *glacial acetic acid*, 10 volumes of *triethylamine*, 20 volumes of *butan-2-ol*, 310 volumes of *acetonitrile* and 690 volumes of *water*, adjusted to pH 3.0 using *orthophosphoric acid*,
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (d). The test is not valid unless the relative retention time with reference to indapamide for 4-chloro-N-(2-methyl-1H-indol-1-yl)-3-sulphamoylbenzamide (indapamide impurity B) is about 1.7; for 4-chloro-3-sulphamoylbenzoic acid is about 0.3.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with test solution, the area of any peak corresponding to indapamide impurity B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any peak corresponding to 4-chloro-3-sulphamoylbenzoic acid is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent). The area of any other secondary peak is not more than five times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of all the impurities is not more than 15 times the area of the principal peak in the chromatograms obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Complies with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Reference solution. Dilute 5.0 ml of 0.025 per cent w/v solution of *indapamide IPRS* in *ethanol (95 per cent)* to 25.0 ml with the mobile phase.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{16}ClN_3O_3S$ in the tablets.

Storage. Store protected from light and moisture at a temperature not exceed 30°.

Indapamide Tablets

Indapamide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indapamide, $C_{16}H_{16}ClN_3O_3S$.

Usual Strengths. 1.5 mg; 2.5 mg.

Identification

A. Shake a quantity of powdered tablets containing about 15 mg of Indapamide with 30.0 ml of *0.2 M sodium hydroxide* in a centrifuge tube for 10 minutes. Centrifuge and transfer the supernatant in a 250 ml separator, add 12 ml of *dilute hydrochloric acid*. Extract the solution twice with 4.0 ml *ether*, filter the extracts through *anhydrous sodium sulphate* and evaporate the ether on a water-bath and dry the crystals at 105° for 1 hour.

Determine by infrared absorption spectrophotometry (2.4.6) of the residue. Compare the spectrum with that obtained with *indapamide IPRS* or with the reference spectrum of indapamide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of phosphate buffer pH 6.8, prepared by dissolving 27.22 g of *potassium dihydrogen phosphate* in about 800 ml of *water*, adjusted to pH 6.8 with 0.2 *M sodium hydroxide*, and diluted to 1000 ml with *water*.

Speed and time. 100 rpm and 45 minutes.

Determine by liquid chromatography (2.4.14),

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve a weighted quantity of *indapamide IPRS* in *methanol* and dilute with dissolution medium to obtain a solution having a known concentration similar to the expected concentration of test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 70 volumes of 0.154 per cent w/v solution of *sodium 1- octanesullphonate*, 1 volume of *glacial acetic acid* and 30 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and test solution.

Calculate the content of $C_{16}H_{16}ClN_3O_3S$.

Q. Not less than 75.0 per cent of the stated amount of $C_{16}H_{16}ClN_3O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light.

Test solution. Disperse 10 intact tablets with 70 ml of *ethanol (95 per cent)* mechanically until the tablets have disintegrated and continue mixing for 2 hours and dilute to 100.0 ml with *ethanol (95 per cent)* and centrifuge. Dilute the supernatant liquid with the mobile phase to obtain a solution containing 0.005 per cent w/v of Indapamide.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of a 0.00025 per cent w/v solution of *indapamide impurity B IPRS* in *ethanol (95 per cent)* to 10.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of a 0.00025 per cent w/v solution of *4-chloro-3-sulphamoylbenzoic acid* in *ethanol (95 per cent)* to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a mixture of 6 volumes of a solution containing 5 per cent w/v of *sodium dodecyl sulphate* and 3 per cent v/v of *glacial acetic acid*, 10 volumes of *triethylamine*, 20 volumes of *butan-2-ol*, 310 volumes of *acetonitrile* and 690 volumes of *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Name	Relative retention time
4-chloro-3-sulphamoylbenzoic acid	0.3
Indapamide	1.0
Indapamide impurity B[1]	1.7

[1]4-chloro-N-(2-methyl-1H-indol-1-yl)-3-sulphamoylbenzamide.

Inject reference solution (a), (b), (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to indapamide impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak corresponding to 4-chloro-3-sulphamoylbenzoic acid is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks other than indapamide impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

Test solution. Disperse one tablet in 1 to 2 ml of *water* and add 25 ml *acetonitrile* with the aid of ultrasound for 20 minutes and dilute to 50 ml with *acetonitrile*. Transfer this solution to centrifuge tube, and centrifuge at 2000 rpm for about 10 minutes. Transfer 10ml of supernatant to a 50 ml volumetric flask, add 3.0 ml of internal standard solution, dilute with *water* to volume and mix.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution.A 0.025 per cent w/v solution of *2- chloroacetophenone* in *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 2.5 mg of Indapamide, disperse in 25 ml *acetonitrile* with the aid of ultrasound for 20 minutes and dilute to 50.0 ml with *acetonitrile*. Transfer this solution to centrifuge tube, and centrifuge at 2000 rpm for about 10 minutes. Transfer 10 ml of supernatant to a 50-ml volumetric flask, add 3.0 ml of internal standard solution, dilute with *water* to volume and mix.

Reference solution. A 0.01 per cent w/v solution of *indapamide IPRS* in *acetonitrile*. Dilute 5.0 ml of the solution, add 3.0 ml of internal standard solution to 50.0 ml with *water*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: 70 volumes of 0.154 per cent w/v solution of *sodium 1- octanesulphonate*, 1 volume of *glacial acetic acid* and 30 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume: 20 µl.

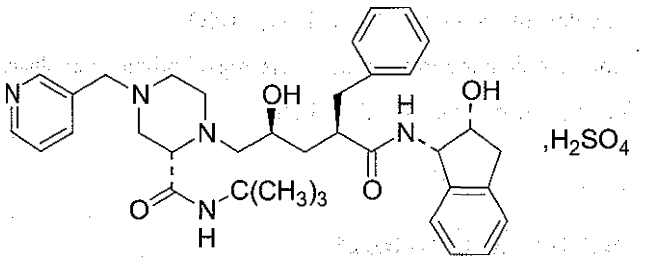
The relative retention time with reference to indapamide for 2- chloroacetophenone is about 1.18.

Inject the reference solution. The test is not valid unless the resolution between the principal peak and the internal standard peak is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent for the principal peak.

Calculate the content of $C_{16}H_{16}ClN_3O_3S$ in the tablets.

Storage. Store protected from light.

Indinavir Sulphate



$C_{36}H_{47}N_5O_4, H_2SO_4$ Mol. Wt. 711.9

Indinavir Sulphate is [1-(1*S*,2*R*),5-(2*S*)]-2,3,5-trideoxy-*N*-(2,3-dihydro-2-hydroxy-1*H*-inden-1-yl)-5-[2-[{1,1-dimethylethyl)amino}carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-D-*erythro*-pentonamide sulphate.

Indinavir Sulphate contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{36}H_{47}N_5O_4,H_2SO_4$, calculated on the anhydrous and ethanol-free basis.

Category. Antiretroviral.

Description. A white or almost white powder; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *indinavir IPRS* or with the reference spectrum of indinavir sulphate.

B. When examined in the range 200 nm to 300 nm (2.4.7), a 0.005 per cent w/v solution in *water* shows an absorption maximum at about 260 nm.

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to indinavir in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 2.8 to 3.2, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). +122° to +129°, determined at about 365 nm in a 1.0 per cent w/v solution in *water*, calculated on the anhydrous and ethanol-free basis.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disolve 0.1 g of the substance under exmination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.0086 per cent w/v solution of *indinavir IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Transfer 15 mg of *indinavir IPRS* to a 50-ml volumetric flask and add 0.1 ml of *5 M hydrochloric acid*. Keep this solution for about 1 hour at room temperature and make up to volume with the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with base deactivated octylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of a solution containing 0.37 per cent w/v of *sodium citrate* and 0.16 per cent w/v of *citric acid*, the pH of which has been adjusted to 5.0 with *1 M sodium hydroxide* or *1 M orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 50 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to indinavir and any impurity at a relative retention time of about 1.4 is not less than 2.

Inject reference solution (a). The test is not valid unless the capacity factor for indinavir peak is not less than 2.0, the

tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5 per cent.

Inject the test solution. Calculate the content of each impurity in the chromatogram obtained with the test solution by comparing with the area of the principal peak obtained with reference solution (a). The content of any individual impurity is not more than 0.1 per cent and the sum of all impurities is not more than 0.5 per cent.

Ethanol. 5.0 to 8.0 per cent.

Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.5 per cent v/v solution of *1-propanol* in *water*.

Test solution. Dissolve 0.4 g of the substance under examination in 50 ml of *water*, add 8.0 ml of the internal standard solution and dilute to 100.0 ml with *water*.

Reference solution. Dilute 1.0 ml of *anhydrous ethanol* to 200.0 ml with *water*. To 2.0 ml of the solution, add 2.0 ml of the internal standard solution and dilute to 25.0 ml with *water*.

Chromatographic system
- a capillary column 30 m × 0.53 mm, packed with fused silica coated with macrogol 20000 (film thickness 1.0 µm),
- temperature:
  column: 35°,
  inlet port at 140° and detector port at 220°,
- flame ionization detector,
- split ratio: 1:10,
- flow rate: 10 ml per minute, using helium as the carrier gas,
- injection volume: 1 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to ethanol and 1- propanol is not less than 5.0.

Inject the reference solution and the test solution.

Calculate the content of ethanol using the ratio of the area of the peak due to ethanol to that of the internal standard. The density of ethanol is 0.79 g per ml.

Sulphate.13.2 to 14.4 per cent w/w, calculated on the anhydrous and ethanol-free basis, determined by the following method. Weigh 0.5 g, dissolve in 50 ml of *dimethylformamide* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0048 g of sulphate.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 60 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.05 per cent w/v solution of *indinavir IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a filtered and degassed mixture of 45 volumes of *acetonitrile* and 55 volumes of a buffer prepared by dissolving 3 g of *phosphoric acid* and 1.7 ml of *dibutylamine* in 900 ml of *water*, adjusted to pH 6.5 with *1 M sodium hydroxide* and making up the volume to 1000.0 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the indinavir peak is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{36}H_{47}N_5O_4, H_2SO_4$.

1 mg of indinavir corresponds to 1.16 mg of indinavir sulphate.

Storage. Store protected from light.

Indinavir Capsules

Indinavir Sulphate Capsules

Indinavir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indinavir, $C_{36}H_{47}N_5O_4$.

Usual strength. The equivalent of 400 mg of indinavir.

Identification

A. Shake a quantity of the contents of the capsules containing 0.1 g of Indinavir Sulphate with 80 ml of *water*, dilute to 100 ml with *water* and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with *water*. When examined in the range 200 nm to 300 nm (2.4.7), the resulting solution shows an absorption maximum at about 260 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to indinavir in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle with sinkers),

Medium. 900 ml of a buffer solution prepared by dissolving 21 g of *citric acid* in 880 ml of *water,* adjusted to pH 3.8 with 50 per cent w/v solution of *sodium hydroxide* and dilute to 1000 ml with *water,*

Speed and time. 50 rpm and 30 minutes. .

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Measure the absorbance of the filtrate, dilute suitably if necessary with the medium, at the maximum at about 260 nm (2.4.7). Calculate the content of indinavir $C_{36}H_{47}N_5O_4$, in the medium from the absorbance obtained from a solution of known concentration of *indinavir IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{36}H_{47}N_5O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of mobile phase A.

Test solution. Disperse a quantity of the mixed powered content of the capsules containing 50 mg of indinavir in 60 ml of the solvent mixture, with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with the solvent mixture, filter.

Reference solution (a). A 0.0005 per cent w/v solution of *indinavir IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.05 per cent w/v of *indinavir IPRS* and 0.005 per cent w/v of *indinavir 4-epimer IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *0.05 M dipotassium hydrogen phosphate,* adjusted to pH 7.5 with *dilute orthophosphoric acid,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
3	80	20
5	65	35
11	65	35
17	30	70
20	30	70
21	80	20
25	80	20

Inject reference solution (a) and (b). The test is not valid unless the resolution between indinavir and indinavir 4-epimer peaks is not less than 1.5, the column efficiency for indinavir peak is not less than 10,000 theoretical plates, the tailing factor is not more than 1.5 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the content of 20 capsules. Disperse a quantity of the mixed powered content of the capsules containing 200 mg of indinavir in 60 ml of the mobile phase, with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with the mobile phase, filter. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *indinavir IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *0.05 M dipotassium hydrogen phosphate,* adjusted to pH 7.5 with *dilute orthophosphoric acid* and 45 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 6000 theoretical

plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

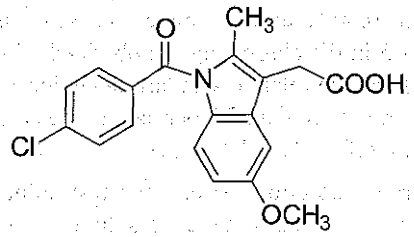
Inject the reference solution and the test solution.

Calculate the content of $C_{36}H_{47}N_5O_4$ in the capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Indomethacin

Indometacin



$C_{19}H_{16}ClNO_4$ Mol. Wt. 357.8

Indomethacin is 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-ylacetic acid.

Indomethacin contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{16}ClNO_4$, calculated on the dried basis.

Category. Antiinflammatory; analgesic.

Description. A white to pale yellow, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *indomethacin IPRS* or with the reference spectrum of indomethacin. Examine the substances in the solid state without recrystallisation.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0025 per cent w/v solution in a mixture of 90 volumes of *methanol* and 10 volumes of *1 M hydrochloric acid* shows an absorption maximum only at about 320 nm; absorbance at about 320 nm, about 0.45.

C. Dissolve 0.1 g in 10 ml of *ethanol (95 per cent)*, heating gently if necessary. To 0.1 ml add 2 ml of a freshly prepared mixture of 1 volume of a 25 per cent w/v solution of *hydroxylamine hydrochloride* and 3 volumes of *2 M sodium hydroxide*. Add 2 ml of *2 M hydrochloric acid* and 1 ml of *ferric chloride solution* and mix; a violet-pink colour develops.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with a suspension of *silica gel HF254* in a 4.68 per cent w/v solution of *sodium dihydrogen phosphate*.

Mobile phase. A mixture of 70 volumes of *ether* and 30 volumes of *light petroleum (50° to 70°)*.

NOTE—Prepare the following solutions immediately before use.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

Reference solution. A 0.01 per cent w/v solution of the substance under examination in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.45 g in 75 ml of *acetone* and titrate under nitrogen with carbonate-free *0.1 M sodium hydroxide* using 0.2 ml of *phenolphthalein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03578 g of $C_{19}H_{16}ClNO_4$.

Storage. Store protected from light.

Indomethacin Capsules

Indomethacin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indomethacin, $C_{19}H_{16}ClNO_4$.

Usual strength. 25 mg.

Identification

A. Shake a quantity of the contents of the capsules containing 0.1 g of Indomethacin with 5 ml of *chloroform*, filter and evaporate the filtrate to dryness. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *indomethacin IPRS* or with the reference spectrum of indomethacin.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 320 nm.

C. Mix a quantity of the contents of the capsules containing 25 mg of Indomethacin with 2 ml of *water* and add 2 ml of *2 M sodium hydroxide*; a bright yellow colour is produced which fades rapidly.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with a suspension of *silica gel HF254* in a 4.68 per cent w/v solution of *sodium dihydrogen phosphate*.

Mobile phase. A mixture of 70 volumes of *ether* and 30 volumes of *light petroleum (50° to 70°)*.

NOTE—Prepare the following solutions immediately before use.

Test solution. Shake a quantity of the contents of the capsules containing 0.1 g of Indomethacin with 5 ml of *chloroform*, filter and use the filtrate.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 750 ml of a freshly prepared mixture of 1 volume of *phosphate buffer pH 7.2* and 4 volumes of *water*,
Speed and time. 100 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute the filtrate suitably with the medium and measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7). Calculate the content of $C_{19}H_{16}ClNO_4$ in the medium from the absorbance obtained from a solution of known concentration of *indomethacin IPRS* in the same medium.

Q. Not less than 80 per cent of the stated amount of $C_{19}H_{16}ClNO_4$.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 50 mg of Indomethacin, add 10 ml of *water* and allow to stand for 10 minutes, with occasional swirling. Add 75 ml of *methanol*, shake well, add sufficient *methanol* to produce 100.0 ml and filter if necessary. To 5.0 ml of the filtrate add sufficient of a mixture of equal volumes of *methanol* and *phosphate buffer pH 7.2* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7). Calculate the content of $C_{19}H_{16}ClNO_4$ taking 193 as the specific absorbance at 320 nm.

Storage. Store protected from moisture.

Indomethacin Suppositories

Indomethacin Suppositories contain Indomethacin in a suitable suppository base.

Indomethacin Suppositories contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of indomethacin, $C_{19}H_{16}ClNO_4$.

Usual strength. 100 mg.

Identification

A. Dissolve a quantity of the powdered suppositories containing 0.1 g of Indomethacin as completely as possible in 50 ml of hot *water*, filter, wash the residue with hot *water* and allow to dry in air. Dissolve the residue in 5 ml of *chloroform* and evaporate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *indomethacin IPRS* or with the reference spectrum of indomethacin.

B. Shake a quantity containing 25 mg of Indomethacin with 5 ml of *water* until the base dissolves; a white suspension is produced. Add 2 ml of *2 M sodium hydroxide*; a bright yellow colour is produced which fades rapidly.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the following solutions freshly.

Test solution (a). Powder or cut into small pieces a suitable number of suppositories, dissolve a quantity containing 0.1 g of Indomethacin in sufficient *methanol* to produce 50 ml.

Reference solution (a). Dilute 3 .0 ml of test solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as Bondapack C18),
- mobile phase: a mixture of 60 volumes of *methanol* and 40 volumes of 0.2 per cent v/v solution of *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 320 nm,
- injection volume: 20 µl.

The sum of the areas of any secondary peaks that elute before the principal peak in the chromatogram obtained with test solution (a) is not greater than the area of the peak in the chromatogram obtained with reference solution (a).

Repeat the procedure but using the following freshly prepared solutions and a detection wavelength of about 240 nm.

Test solution (b). Dilute 10.0 ml of test solution (a) to 20.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.001 per cent w/v of *4-chlorobenzoic acid* in the mobile phase.

In the chromatogram obtained with test solution (b) the sum of the areas of any secondary peaks that elute before the principal peak, other than those determined in test solution (a), is not greater than the area of the peak in the chromatogram obtained with reference solution (b).

The column efficiency, determined using the principal peak in the chromatogram obtained with reference solution (a), should be not less than 7500 theoretical plates per metre.

Disintegration (2.5.1). Use a weighed suppository and *phosphate buffer pH 6.8* in place of *water* and operate the apparatus for 90 minutes. At the end of this period remove the suppository, dry with filter paper and weigh. Repeat the operation with two further weighed suppositories. Not less than 75 per cent of each suppository is dissolved.

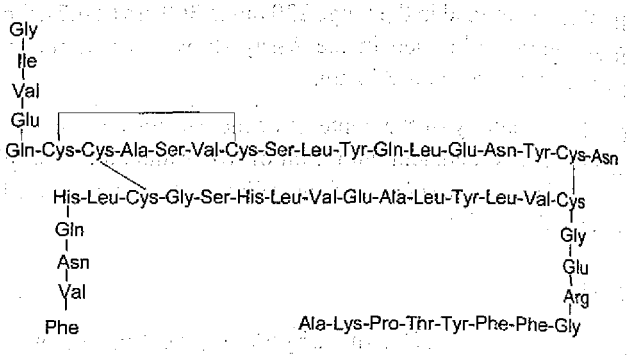
Other tests. Comply with the tests stated under Suppositories.

Assay. Weigh 10 suppositories and powder or cut into small pieces. Disperse a quantity of the powder or small pieces containing about 0.1 g of Indomethacin, add 50 ml of *methanol*, shake until the dispersion is complete and, if necessary, filter. To 2.0 ml add sufficient of a mixture of equal volumes of *methanol* and *phosphate buffer pH 7.2* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7). Calculate the content of $C_{19}H_{16}ClNO_4$ taking 193 as the specific absorbance at 320 nm.

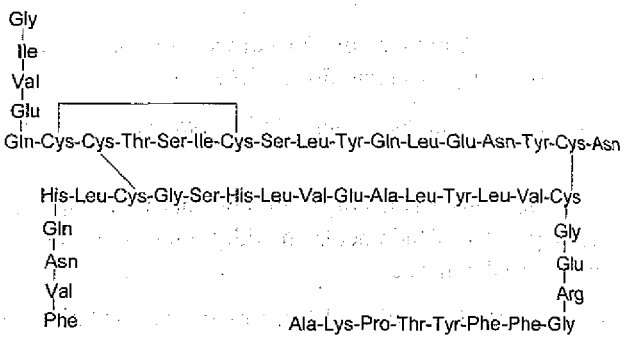
Storage. Store protected from moisture at a temperature not exceeding 30°.

Insulin

Crystalline Insulin



$C_{256}H_{381}N_{65}O_{76}S_6$ (porcine) Mol. Wt. 5777.6



$C_{254}H_{377}N_{65}O_{75}S_6$ (bovine) Mol. Wt. 5733.5

Insulin is the specific natural antidiabetic principle obtained from the pancreas of either the pig or the ox and purified.

Insulin contains not less than 26.5 IU per mg of porcine insulin, $C_{256}H_{381}N_{65}O_{76}S_6$, or of bovine insulin, $C_{254}H_{377}N_{65}O_{75}S_6$, as appropriate, calculated on the dried basis.

Category. Hypoglycaemic.

Description. A white or almost white powder.

Identification

A. In the Assay, the principal peak due to insulin in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the appropriate reference solution.

B. Determine by liquid chromatography (2.4.14), the peptide fragments, using the following peptide mapping procedure.

Test solution. Prepare a 0.2 per cent w/v solution of the substance under examination in *0.01M hydrochloric acid* and transfer 500 µl of the solution to a stoppered clean tube. Add 2.0 ml of *HEPES buffer solution pH 7.5* and 400 µl of a 0.1 per cent w/v solution of *Staphylococcus aureus strain V8*

protease. Close the tube and incubate at 25° for 6 hours. Stop the reaction by adding 2.9 ml of *sulphate buffer solution pH 2.0*.

Reference solution. Prepare in the same manner as for the test solution but using as appropriate, *porcine insulin IPRS* or *bovine insulin IPRS* or *human insulin IPRS* in place of the substance under examination.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane silica gel (3 µm) with a pore size of 8 nm,
- column temperature: 40°,
- mobile phase: A. a mixture of 100 ml of *acetonitrile*, 200 ml of *sulphate buffer solution pH 2.0* and 700 ml of *water*,

  B. a mixture of 200 ml of *sulphate buffer solution pH 2.0*, 400 ml of *acetonitrile* and 400 ml of *water*,
- a gradient programme using the conditions given below
- flow rate: 1 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
60	30	70
65	0	100
70	0	100
71	90	10
80	90	10

At initial conditions equilibrate the column for at least 15 minutes. Carry out a blank run using the above-mentioned gradient.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 for the chromatograms obtained with the reference solution for the peaks due to fragments II and III and the resolution factor between the peaks due to fragments II and III is not less than 1.9 for porcine and bovine insulin.

Inject the reference solution and the test solution. The chromatograms obtained with the test and reference solution are qualitatively similar. In the chromatogram obtained with the reference solution identify the peaks due to digest fragments I, II and III.

The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

NOTE —The retention time of fragment III is the same for bovine insulin and for porcine insulin. The retention times of fragments II and IV are the same for all insulins.

Tests

Light absorption (2.4.7). Absorbance of a 0.05 per cent w/v solution in *0.01 M hydrochloric acid* at the maximum at about 276 nm, 0.48 to 0.56.

Other tests. Comply with the tests for Impurities with molecular masses greater than that of insulin, Related proteins and Total zinc stated under Insulin Preparations.

Sulphated ash (2.3.18). Not more than 2.5 per cent, calculated on the dried basis, determined on 0.2 g.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 0.2 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Determine as described under Assay of Insulins (2.3.46).

Test solution. Dissolve a suitable quantity of the substance under examination in *0.01 M hydrochloric acid* to obtain a concentration of 4.0 mg per ml.

Insulin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 20 Endotoxin Units per mg.

Storage. Store protected from light, at a temperature not exceeding –20° until released by the manufacturer. When thawed, it should be stored in a refrigerator (2° to 8°) and used for the manufacture of preparations within a short period of time.

Labelling. The label states (1) the animal source or sources of the insulin; (2) where applicable, that the material is free from bacterial endotoxins; (3) the storage conditions.

NOTE — 0.0345 mg of porcine or 0.0342 mg of bovine insulin is equivalent to 1 Unit of insulin.

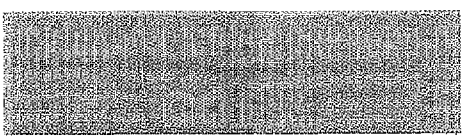
Biphasic Insulin Injection

Biphasic Insulin Injection is a sterile suspension of crystals containing bovine insulin in a solution of porcine insulin.

Biphasic Insulin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of IU of Insulin.

Usual strength. 40 IU per ml (30: 70).

Description. A white suspension. When examined under a microscope, the majority of the particles appear as rhombohedral crystals, with a maximum dimension of the crystals greater than 10 µm but rarely exceeding 40 µm.



Identification

In the chromatograms obtained in the Assay the position of the peaks due to the two insulins in the chromatogram obtained with the test solution correspond to those of the principal peaks in the chromatogram obtained with the appropriate reference solution.

Tests

pH (2.4.24). 6.6 to 7.2.

Total zinc. 26.0 µg to 37.5 µg per 100 IU of insulin, determined by either of the methods stated under Insulin Preparations.

Insulin in the supernatant. 22.0 per cent to 28.0 per cent of insulin in solution, determined as stated under Insulin Preparations.

Other tests. Comply with the tests stated under Insulin Preparations.

Assay. Determine as described under Assay of Insulins (2.3.46).

Test solution. To 10 ml of the preparation under examination add 40 µl of *5 M hydrochloric acid*, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 40 IU per ml.

Insulin Zinc Suspension

I.Z.S; Insulin Zinc Suspension (Mixed); I.Z.S (Mixed); Insulin Lente

Insulin Zinc Suspension is a sterile, buffered suspension of Insulin in the form of a complex obtained by the addition of zinc chloride to insulin in a manner such that the insulin is in a form insoluble in *water*. It may be prepared by mixing aseptically about 3 volumes of Insulin Zinc Suspension (Amorphous) and about 7 volumes of Insulin Zinc Suspension (Crystalline) and distributing the mixture aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Insulin Zinc Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of IU of Insulin.

Usual strengths. 40 IU per ml; 80 IU per ml.

Description. A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, the majority of the particles in the suspension are seen as rhombohedral crystals, with a maximum dimension greater than 10 µm but rarely exceeding 40 µm; a considerable number of particles have no uniform shape and do not exceed 2 µm in maximum dimension.

Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with test solution has a retention time similar to that of the principal peak in the chromatogram obtained with reference solution (a), (b) or (c) as appropriate.

Tests

pH (2.4.24). 6.9 to 7.5.

Total zinc. Not more than 0.0095 per cent w/v (for preparations containing 40 IU per ml) and not more than 0.014 per cent w/v (for preparations containing 80 IU per ml), determined by the following method. Take a volume of the well-shaken suspension containing 200 IU of insulin and add 1 ml of *0.1M hydrochloric acid*, 10 ml of *alkaline borate buffer pH 9.0*, 1 ml of *0.1M sodium hydroxide*, 2 ml of a 0.0009 per cent w/v solution of *trypsin* in *0.01M hydrochloric acid*. Mix, allow to stand for 10 minutes and add 3 ml of *zincon solution* and sufficient *water* to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of *water* instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of *zinc sulphate solution* and 6 volumes of *water*.

Zinc in solution. Not more than 70 per cent of the total zinc (for preparations containing 40 IU per ml) and not more than 55 per cent of the total zinc (for preparations containing 80 IU per ml), determined by the method described in the test for Total zinc, using 1 ml of the clear supernatant liquid obtained by centrifuging and beginning at the words "add 1 ml of *0.1M hydrochloric acid,.....*".

Insulin extractable with buffered acetone solution. 27 per cent to 40 per cent, determined by the following method. Centrifuge a volume containing 400 IU and reject the supernatant liquid. Suspend the residue in 3.3 ml of *water*, add 6.6 ml of *buffered acetone solution*, stir for 3 minutes and again centrifuge. Transfer the supernatant liquid as completely as possible to a long-necked, round-bottomed flask, add 0.3 g of *nitrogen-free mercuric oxide*, 3 g of *anhydrous sodium sulphate*, and 6 ml of *nitrogen-free sulphuric acid*, heat over a low flame until the liquid is colourless and boil for a further 30 minutes. Allow to cool, dilute carefully with *water*, add 1 g of *zinc powder*, shake and allow to stand for 10 minutes. Add an excess of *sodium hydroxide solution*, immediately connect the flask to an ammonia distillation apparatus, mix the contents and distil the liberated ammonia into 20 ml of *0.005 M sulphuric acid* prepared with *carbon dioxide-free water*. Rinse the condenser

tube into the flask containing the distillate, add sufficient *carbon dioxide-free water* to produce a total volume of about 50 ml and titrate the excess of *sulphuric acid* with *0.01 M sodium hydroxide* to pH 6.0, using a glass electrode. Centrifuge a further volume containing 400 IU and reject the supernatant liquid. Dissolve the residue in 10 ml of a 5 per cent w/v solution of *nitrogen-free sulphuric acid*, transfer to a long-necked, round-bottomed flask, and repeat the procedure described above beginning at the words "add 0.3 g of *nitrogen-free mercuric oxide*,....". Calculate the percentage of insulin extractable with the buffered acetone solution from the formula $100A/B$, where A is the volume of *0.005 M sulphuric acid* used in the first determination and B is the volume used in the second determination.

The result of the test is not valid unless in carrying out the first determination omitting the insulin preparation, not more than 0.2 ml of *0.005 M sulphuric acid* is required.

Insulin in solution. Determine by liquid chromatography (2.4.14).

Test solution. For preparations containing 100 IU per ml Centrifuge a well-shaken suspension of the preparation under examination, transfer 2.0 ml of the supernatant liquid to a 5 ml volumetric flask, dilute to volume with *0.03 M hydrochloric acid* and mix well.

Reference solution (a). Prepare as test solution but using 2.5 ml of the supernatant liquid in place of 2.0 ml.

Reference solution (b). Use 5 ml of the supernatant liquid.

Reference solution (c). Weigh 4.5 mg of *bovine insulin IPRS* into a 100-ml volumetric flask containing 50 ml of *0.025 M hydrochloric acid*, dissolve by shaking for 5 minutes, dilute to volume with *0.025 M hydrochloric acid* and mix to obtain a solution containing approximately 1 Unit per ml.

Use the chromatographic system as described under Assay.

The area of the peak due to insulin in the chromatogram obtained with test solution, reference solution (a) or reference (b), as the case may be, is not more than that of the principal peak in the chromatogram obtained with reference solution (c).

Bacterial endotoxins (2.2.3). Not more than 80 Endotoxin Units per 100 Units of insulin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. To 10 ml of the preparation under examination add 40 ml of *5 M hydrochloric acid*, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 20 IU per ml.

Reference solution (a). A 0.08 per cent w/v of *bovine insulin IPRS* in *0.025 M hydrochloric acid*.

Reference solution (b). A 0.08 per cent w/v of *porcine insulin IPRS* in *0.025 M hydrochloric acid*.

Reference solution (c). A solution containing 0.04 per cent w/v of *bovine insulin IPRS* and 0.04 per cent w/v of *porcine insulin IPRS* in *0.025 M hydrochloric acid* for a preparation containing both bovine and pork insulin.

Reference solution (d). A 0.04 per cent w/v each of *human insulin IPRS* and *porcine insulin IPRS* in *0.025 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Ultrasphere ODS),
- column temperature. 45°,
- mobile phase. a mixture of 72.5 volumes of *0.1 M sodium dihydrogen phosphate* adjusted to pH 2.0 with *orthophosphoric acid* and 27.5 volumes of *acetonitrile*,
- flow rate 1 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 50 µl.

The test is not valid unless the resolution factor between the peaks corresponding to human insulin and porcine insulin is at least 1.2 in the chromatogram obtained with reference solution (d). If necessary, adjust the concentration of *acetonitrile* in the mobile phase by slight decrease or increase until the required resolution is obtained. In the chromatogram obtained with reference solution (d) the two principal peaks, in order of emergence, are due to human insulin and porcine insulin and any smaller peaks appearing immediately following each of the principal peaks are due to the corresponding monodesamido derivatives.

Inject either of reference solution (a), (b) and (c). The test is not valid unless the relative standard deviation of the area of the principal peak is not more than 2.0 per cent.

Inject the test solution. If necessary, make further adjustments in the composition of the mobile phase so that the antimicrobial preservatives present in test solution are well separated from insulin and show shorter retention times. A small reduction in the concentration of *acetonitrile* increases the retention time of insulin peak relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution, wash the column with a mixture of equal volumes of *acetonitrile* and *water* for a sufficient time in order to elute any interfering substances before injecting the next solution.

Calculate the content of insulin from the area of the peaks due to the bovine, porcine or human insulin and that of any peak due to the monodesamido derivative of the insulin from the

declared content of insulin in *bovine insulin IPRS, porcine insulin IPRS* or *human insulin IPRS*, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of any peak due to the desamido derivative of either insulin.

Storage. Store in multiple dose containers at a temperature between 2° and 8°. It should not be allowed to freeze.

Labelling. The label states (1) the strength in terms of the number of IU per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

Insulin Zinc Suspension (Amorphous)

Amorph. I.Z.S.; Prompt Insulin Zinc Suspension

Insulin Zinc Suspension (Amorphous) is a sterile, buffered suspension of Insulin in the form of a complex obtained by the addition of zinc chloride to insulin in a manner such that the solid phase of the suspension is amorphous. It may be prepared by adding aseptically to crystalline insulin having a potency not less than 23 IU per mg, calculated on the dried basis, a suitable quantity of zinc chloride, an appropriate amount of a suitable substance to render the preparation isotonic with blood and a sufficient quantity of a suitable bactericide. It is distributed aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Insulin Zinc Suspension (Amorphous) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of IU of Insulin.

Category. Hypoglycaemic.

Usual strengths. 40 IU per ml; 80 IU per ml.

Description. A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, the particles in the suspension are seen to have no uniform shape and rarely exceed 2 µm in maximum dimension.

Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with test solution has a retention time similar to that of the principal peak in the chromatogram obtained with reference solution (a), (b) or (c) as appropriate.

Tests

pH (2.4.24). 6.9 to 7.5.

Total zinc. Not more than 0.0095 per cent w/v (for preparations containing 40 IU per ml) and not more than 0.014 per cent w/v (for preparations containing 80 IU per ml), determined by the following method. Take a volume of the well-shaken suspension containing 200 IU of insulin and add 1 ml of *0.1 M hydrochloric acid*, 10 ml of *alkaline borate buffer pH 9.0*, 1 ml of *0.1 M sodium hydroxide*, 2 ml of a 0.0009 per cent w/v solution of *trypsin* in *0.01 M hydrochloric acid*. Mix, allow to stand for 10 minutes and add 3 ml of *zincon solution* and sufficient *water* to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of *water* instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of *zinc sulphate solution* and 6 volumes of *water*.

Zinc in solution. Not more than 70 per cent of the total zinc (for preparations containing 40 IU per ml) and not more than 55 per cent of the total zinc (for preparations containing 80 IU per ml), determined by the method described in the test for Total zinc, using 1 ml of the clear supernatant liquid obtained by centrifuging and beginning at the words "add 1 ml of *0.1 M hydrochloric acid*,.....".

Insulin in solution. Determine by liquid chromatography (2.4.14).

Test solution. (for preparations containing 100 IU per ml) centrifuge a well-shaken suspension of the preparation under examination, transfer 2.0 ml of the supernatant liquid to a 5 ml volumetric flask, dilute to volume with *0.03 M hydrochloric acid* and mix well.

Reference solution (a). Prepare as test solution but using 2.5 ml of the supernatant liquid in place of 2.0 ml.

Reference solution (b). Use 5 ml of the supernatant liquid.

Reference solution (c). Weigh 4.5 mg of *bovine insulin IPRS* into a 100-ml volumetric flask containing 50 ml of *0.025 M hydrochloric acid*, dissolve by shaking for 5 minutes, dilute to volume with *0.025 M hydrochloric acid* and mix to obtain a solution containing approximately 1 Unit per ml.

Use the chromatographic system as described under Assay.

The area of the peak due to insulin in the chromatogram obtained with test solution, reference solution (a) or (b), as the case may be, is not more than that of the principal peak in the chromatogram obtained with reference solution (c).

Bacterial endotoxins (2.2.3). Not more than 80 Endotoxin Units per 100 Units of insulin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. To 10 ml of the preparation under examination add 40 ml of *5 M hydrochloric acid*, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 20 IU per ml.

Reference solution (a). A 0.08 per cent w/v of *bovine insulin IPRS* in *0.025 M hydrochloric acid*.

Reference solution (b). A 0.08 per cent w/v of *porcine insulin IPRS* in *0.025 M hydrochloric acid*.

Reference solution (c). A solution containing 0.04 per cent w/v of *bovine insulin IPRS* and 0.04 per cent w/v of *porcine insulin IPRS* in *0.025 M hydrochloric acid* for a preparation containing both bovine and pork insulin.

Reference solution (d). A 0.04 per cent w/v each of *human insulin IPRS* and *porcine insulin IPRS* in *0.025 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column. temperature 45°,
- mobile phase. a mixture of 72.5 volumes of *0.1 M sodium dihydrogen phosphate* adjusted to pH 2.0 with *phosphoric acid* and 27.5 volumes of *acetonitrile*,
- flow rate 1 ml per minute.
- spectrophotometer set at 214 nm,
- injection volume: 50 µl.

The test is not valid unless the resolution factor between the peaks corresponding to human insulin and porcine insulin is at least 1.2 in the chromatogram obtained with reference solution (d). If necessary, adjust the concentration of *acetonitrile* in the mobile phase by slight decrease or increase until the required resolution is obtained. In the chromatogram obtained with reference solution (d) the two principal peaks, in order of emergence, are due to human insulin and porcine insulin and any smaller peaks appearing immediately following each of the principal peaks are due to the corresponding monodesamido derivatives.

Inject either of reference solution (a), (b) and (c). The test is not valid unless the relative standard deviation of the area of the principal peak is not more than 2.0 per cent.

Inject the test solution. If necessary, make further adjustments in the composition of the mobile phase so that the antimicrobial preservatives present in test solution are well separated from insulin and show shorter retention times. A small reduction in the concentration of acetonitrile increases the retention time of insulin peak relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution, wash the column with a mixture of equal volumes of *acetonitrile* and *water* for a sufficient time in order to elute any interfering substances before injecting the next solution.

Calculate the content of insulin from the area of the peaks due to the bovine, porcine or human insulin and that of any peak due to the monodesamido derivative of the insulin from the declared content of insulin in *bovine insulin IPRS, porcine insulin IPRS* or *human insulin IPRS*, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of any peak due to the desamido derivative of either insulin.

Storage. Store in multiple dose containers at a temperature between 2° and 8°. It should not be allowed to freeze.

Labelling. The label states (1) the strength in terms of the number of IU per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

Insulin Zinc Suspension (Crystalline)

Cryst. I.Z.S.; Extended Insulin Zinc Suspension

Insulin Zinc Suspension (Crystalline) is a sterile, buffered suspension of Insulin in the form of a complex obtained by the addition of zinc chloride to insulin in a manner such that the insulin is in the form of crystals insoluble in *water*. It may be prepared by adding aseptically to crystalline insulin having a potency not less than 23 IU per mg, calculated with reference to the dried substance, a suitable quantity of zinc chloride, an appropriate amount of a suitable substance to render the preparation isotonic with blood and a sufficient quantity of a suitable bactericide. The solution is partially neutralised to allow crystallisation to occur and the pH of the crystalline suspension is adjusted to about 7.2. The suspension is distributed aseptically into sterile containers which are then sealed so as to exclude micro-organisms.

Insulin Zinc Suspension (Crystalline) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of IU of Insulin.

Category. Hypoglycaemic.

Usual strengths. 40 IU per ml; 80 IU per ml.

Description. A white suspension which on standing deposits a white sediment and leaves an almost colourless supernatant liquid. The sediment is readily resuspended on gentle shaking. When examined under a microscope, the particles in the suspension are seen to be rhombohedral crystals, the majority

having a maximum dimension greater than 10 µm but rarely exceeding 40 µm.

Identification

In the Assay, the principal peak due to insulin in the chromatogram obtained with test solution has a retention time similar to that of the principal peak in the chromatogram obtained with reference solution (a), (b) or (c) as appropriate.

Tests

pH (2.4.24). 6.9 to 7.5.

Total zinc. Not more than 0.0095 per cent w/v (for preparations containing 40 IU per ml) and not more than 0.014 per cent w/v (for preparations containing 80 IU per ml), determined by the following method. Take a volume of the well-shaken suspension containing 200 IU of insulin and add 1 ml of *0.1 M hydrochloric acid*, 10 ml of *alkaline borate buffer pH 9.0*, 1 ml of *0.1 M sodium hydroxide*, 2 ml of a 0.0009 per cent w/v solution of *trypsin* in *0.01 M hydrochloric acid*. Mix, allow to stand for 10 minutes and add 3 ml of *zincon solution* and sufficient *water* to produce 50 ml. Allow to stand for 1 hour and measure the absorbance of the resulting solution at about 620 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of *water* instead of the substance under examination in a similar manner. Calculate the content of zinc from the absorbance obtained by repeating the procedure using a suitable aliquot of a mixture of 4 volumes of *zinc sulphate solution* and 6 volumes of *water*.

Zinc in solution. Not more than 70 per cent of the total zinc (for preparations containing 40 IU per ml) and not more than 55 per cent of the total zinc (for preparations containing 80 IU per ml), determined by the method described in the test for Total zinc, using 1 ml of the clear supernatant liquid obtained by centrifuging and beginning at the words "add 1 ml of *0.1 M hydrochloric acid*,.....".

Insulin extractable with buffered acetone solution. Not more than 15 per cent, determined by the following method. Centrifuge a volume containing 400 IU and reject the supernatant liquid. Suspend the residue in 3.3 ml of *water*, add 6.6 ml of *buffered acetone solution*, stir for 3 minutes and again centrifuge. Transfer the supernatant liquid as completely as possible to a long-necked, round-bottomed flask, add 0.3 g of *nitrogen-free mercuric oxide*, 3 g of *anhydrous sodium sulphate*, and 6 ml of *nitrogen-free sulphuric acid*, heat over a low flame until the liquid is colourless and boil for a further 30 minutes. Allow to cool, dilute carefully with *water*, add 1 g of *zinc powder*, shake and allow to stand for 10 minutes. Add an excess of *sodium hydroxide solution*, immediately connect the flask to an ammonia distillation apparatus, mix the contents and distil the liberated ammonia into 20 ml of *0.005 M sulphuric acid* prepared with *carbon dioxide-free water*. Rinse the condenser tube into the flask containing the distillate, add sufficient *carbon dioxide-free water* to produce a total volume of about 50 ml and titrate the excess of sulphuric acid with *0.01 M sodium hydroxide* to pH 6.0, using a glass electrode. Centrifuge a further volume containing 400 IU and reject the supernatant liquid. Dissolve the residue in 10 ml of a 5 per cent w/v solution of *nitrogen-free sulphuric acid*, transfer to a long-necked, round-bottomed flask, and repeat the procedure described above beginning at the words "add 0.3 g of *nitrogen-free mercuric oxide*,.....". Calculate the percentage of insulin extractable with the buffered acetone solution from the formula $100A/B$, where A is the volume of *0.005 M sulphuric acid* used in the first determination and B is the volume used in the second determination.

The result of the test is not valid unless in carrying out the first determination omitting the insulin preparation, not more than 0.2 ml of *0.005 M sulphuric acid* is required.

Insulin in solution. Determine by liquid chromatography (2.4.14).

Test solution. For preparations containing 100 IU per ml. Centrifuge a well-shaken suspension of the preparation under examination, transfer 2.0 ml of the supernatant liquid to a 5 ml volumetric flask, dilute to volume with *0.03 M hydrochloric acid* and mix well.

Reference solution (a). Prepare as the test solution but using 2.5 ml of the supernatant liquid in place of 2.0 ml.

Reference solution (b). Use 5 ml of the supernatant liquid.

Reference solution (c). Weigh 4.5 mg of *bovine insulin IPRS* into a 100-ml volumetric flask containing 50 ml of *0.025 M hydrochloric acid*, dissolve by shaking for 5 minutes, dilute to volume with *0.025 M hydrochloric acid* and mix to obtain a solution containing approximately 1 Unit per ml.

Use the chromatographic system as described under Assay.

The area of the peak due to insulin in the chromatogram obtained with test solution, reference solution (a) or reference (b), as the case may be, is not more than that of the principal peak in the chromatogram obtained with reference solution (c).

Bacterial endotoxins (2.2.3). Not more than 80 Endotoxin Units per 100 Units of insulin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. To 10 ml of the preparation under examination add 40 ml of *5 M hydrochloric acid*, mix well, allow to stand for 1 hour to ensure solution of the sediment and dilute with *0.03 M hydrochloric acid* to obtain a solution containing 20 IU per ml.

Reference solution (a). A 0.08 per cent w/v of *bovine insulin IPRS* in *0.025 M hydrochloric acid*.

Reference solution (b). A 0.08 per cent w/v of *porcine insulin IPRS* in *0.025 M hydrochloric acid*.

Reference solution (c). A solution containing 0.04 per cent w/v of *bovine insulin IPRS* and 0.04 per cent w/v of *porcine insulin IPRS* in *0.025 M hydrochloric acid* for a preparation containing both bovine and pork insulin.

Reference solution (d). A 0.04 per cent w/v each of *human insulin IPRS* and *porcine insulin IPRS* in *0.025 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane chemically bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase. a mixture of 72.5 volumes of *0.1 M sodium dihydrogen phosphate* adjusted to pH 2.0 with *orthophosphoric acid* and 27.5 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 50 µl.

The test is not valid unless the resolution factor between the peaks corresponding to human insulin and porcine insulin is at least 1.2 in the chromatogram obtained with reference solution (d). If necessary, adjust the concentration of *acetonitrile* in the mobile phase by slight decrease or increase until the required resolution is obtained. In the chromatogram obtained with reference solution (d) the two principal peaks, in order of emergence, are due to human insulin and porcine insulin and any smaller peaks appearing immediately following each of the principal peaks are due to the corresponding monodesamido derivatives.

Inject either of reference solution (a), (b) and (c). The test is not valid unless the relative standard deviation of the area of the principal peak is not more than 2.0 per cent.

Inject the test solution. If necessary, make further adjustments in the composition of the mobile phase so that the antimicrobial preservatives present in test solution are well separated from insulin and show shorter retention times. A small reduction in the concentration of *acetonitrile* increases the retention time of insulin peak relatively more than those of the preservatives. If necessary, after having carried out the chromatography of a solution, wash the column with a mixture of equal volumes of *acetonitrile* and *water* for a sufficient time in order to elute any interfering substances before injecting the next solution.

Calculate the content of insulin from the area of the peaks due to the bovine, porcine or human insulin and that of any peak due to the monodesamido derivative of the insulin from the declared content of insulin in *bovine insulin IPRS*, *porcine insulin IPRS* or *human insulin IPRS*, as appropriate. For preparations containing both bovine and porcine insulin use the sum of the areas of both the bovine and porcine insulin peaks and of any peak due to the desamido derivative of either insulin.

Storage. Store in multiple dose containers at a temperature between 2° and 8°. It should not be allowed to freeze.

Labelling. The label states (1) the strength in terms of the number of IU per ml; (2) the animal source or sources of the insulin; (3) that the preparation should not be allowed to freeze; (4) that the container should be gently shaken before a dose is withdrawn; (5) the storage conditions.

Invert Sugar Injection

Invert Sugar Injection is a sterile solution of a mixture of equal amounts of Dextrose and Fructose in Water for Injections, or an equivalent sterile solution produced by the hydrolysis of Sucrose in Water for Injections. It contains no antimicrobial agent.

Invert Sugar Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the labelled amount of $C_6H_{12}O_6$.

Usual strengths. 5, 10, and 20 per cent w/v.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

Tests

pH (2.4.24). 3.0 to 6.5.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of invert sugar to 500.0 ml with *water* and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25.

Heavy metals (2.3.13). A solution prepared by evaporating a volume containing 4.0 g of invert sugar to 10 ml and adding 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (5 ppm).

Chlorides (2.3.12). 2 ml of the injection complies with the limit test for chlorides (125 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Completeness of inversion

NOTE — Invert Sugar Injection that is produced by mixing Dextrose and Fructose is exempt from this test.

Determine by liquid chromatography (2.4.14).

Test solution. Transfer a measured volume of the preparation under examination containing about 2.5 g of invert sugar to a 100-ml volumetric flask, dilute to volume with *water* and mix.

Reference solution. Prepare a solution in *water* containing known concentrations of about 0.25 mg of sucrose and about 12.5 mg of dextrose per ml.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the calcium form (9 µm),
- column temperature: 40°,
- mobile phase: *water*,
- flow rate: 1 ml per minute,
- refractive index detector,
- injection volume: 20 µl.

Inject the reference solution. The sucrose elutes first and the peak is baseline separated from the dextrose peak. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution and measure the responses for the sucrose peak. Calculate the content of sucrose in the volume taken of the preparation under examination. Not more than 1.5 per cent of the quantity of invert sugar in the volume taken of the preparation under examination, based on the value stated on the label, is found.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Transfer 50.0 ml of *cupri-tartaric solution* into a 400 ml beaker, add 48 ml of *water*, mix and add 2.0 ml of the preparation under examination that has been diluted quantitatively with *water*, if necessary, to a 5.0 per cent concentration. Cover the beaker with a watch glass, heat the solution, regulating the heat so that boiling begins in 4 minutes and continue boiling for 2 minutes. Filter the hot solution at once through a tared porcelain filtering crucible, wash the precipitate with water maintained at 60°, then with 10 ml of *ethanol (95 per cent)*. Dry at 105° to constant weight. Carry out a blank determination and make any necessary correction. The corrected weight of the precipitate so obtained is not less than 204.0 mg and not more than 224.4 mg.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) whether it is produced by hydrolysis of Sucrose or by mixing Dextrose and Fructose; (2) the strength as the percentage w/v of invert sugar; (3) total osmolar concentration in mOsmol per litre; (4) that the injection should not be used if it contains visible particles.

Invert Sugar and Sodium Chloride Injection

Sodium Chloride and Invert Sugar Intravenous Infusion

Invert Sugar and Sodium Chloride Injection is a sterile solution of a mixture of equal amounts of Dextrose and Fructose in Water for Injections to which the required amount of Sodium Chloride is added. Invert sugar may be prepared by acid hydrolysis of Sucrose.

Invert Sugar and Sodium Chloride Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of sodium chloride, NaCl, and invert sugar, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Usual strengths. Injections containing the following amounts of Sodium Chloride, NaCl, and invert sugar, $C_6H_{12}O_6$.

Sodium Chloride (NaCl) (per cent w/v)	Invert Sugar ($C_6H_{12}O_6$) (per cent w/v)
0.45	5
0.45	10
0.90	5
0.90	10

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. It gives reaction (A) of chlorides and reaction (B) of sodium salts (2.3.1).

Tests

pH (2.4.24). 3.0 to 6.5.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of invert sugar to 500.0 ml with *water*

and measure the absorbance (2.4.7) of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25.

Completeness of inversion

NOTE — Invert Sugar and Sodium Chloride Injection that is produced by mixing Dextrose and Fructose is exempt from this test.

Determine by liquid chromatography (2.4.14).

Test solution. Transfer a measured volume of the preparation under examination containing about 2.5 g of invert sugar to a 100-ml volumetric flask, dilute to volume with *water* and mix.

Reference solution. Prepare a solution in *water* containing known concentrations of 0.25 mg of sucrose and 12.5 mg of dextrose per ml.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the calcium form (9 µm),
- column temperature: 40°,
- mobile phase: *water*,
- flow rate: 1 ml per minute,
- refractive index detector,
- injection volume: 20 µl.

Inject the reference solution. The sucrose elutes first and the peak is baseline separated from the dextrose peak. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution and measure the responses for the sucrose peak. Calculate the content of sucrose in the volume taken of the preparation under examination. Not more than 1.5 per cent of the quantity of invert sugar in the volume taken of the preparation under examination, based on the value stated on the label, is found.

Heavy metals (2.3.13). A solution prepared by evaporating a volume containing 4.0 g of invert sugar to 10 ml and adding 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml complies with the limit test for heavy metals, Method A (5 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *For sodium chloride* — Titrate a measured volume containing about 0.1 g of Sodium Chloride with *0.1 M silver nitrate* using *potassium chromate solution* as indicator. 1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

For invert sugar — Transfer 50.0 ml of *cupri-tartaric solution* into a 400-ml beaker, add 48 ml of *water*, mix and add 2.0 ml of the preparation under examination that has been diluted quantitatively with *water*, if necessary, to a 5.0 per cent concentration. Cover the beaker with a watch glass, heat the solution, regulating the heat so that boiling begins in 4 minutes and continue boiling for 2 minutes. Filter the hot solution at once through a tared porcelain filtering crucible, wash the precipitate with water maintained at 60°, then with 10 ml of *ethanol (95 per cent)*. Dry at 105° to constant weight. Carry out a blank determination and make any necessary correction. The corrected weight of the precipitate so obtained is not less than 204.0 mg and not more than 224.4 mg.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) whether it is produced by hydrolysis of Sucrose or by mixing Dextrose and Fructose; .(2) the strength as the percentage w/v of sodium chloride and invert sugar; (3) total osmolar concentration in mOsmol per litre; (4) approximate concentrations, in millimoles per litre, of the sodium and chloride ions; (5) that the injection should not be used if it contains visible particles.

Invert Syrup

Invert Syrup is a mixture of glucose and fructose prepared by hydrolysing a 66.7 per cent w/w solution of Sucrose with a suitable mineral acid, such as hydrochloric acid and neutralising the resulting solution using calcium carbonate or sodium carbonate. The degree of inversion is at least 95 per cent.

Invert Syrup contains not less than 67.0 per cent w/w of the stated amount of reducing sugars, expressed as invert sugar.

Category. Sweetener.

Description. A clear, colourless to pale straw-coloured syrupy liquid. Miscible with *water*, producing a clear solution; it dissolves in *ethanol (95 per cent)* with the formation of an insoluble residue.

Identification

A. Heat 1 g with 10 ml of *water* and 5 ml of *cupri-tartaric solution*, a red precipitate is formed.

B. A solution in *water* is laevorotatory.

Tests

pH (2.4.24). 5.0 to 6.0.

Arsenic (2.3.10). To 4.0 g, add 50 ml of *water* and 10 ml of *brominated hydrochloric acid*, allow to stand for 5 minutes and remove the excess of bromine by adding *tin(II) chloride solution AsT* and dilute to 100 ml with *water*. 25 ml of the solution complies with the limit test for arsenic (1 ppm), using 1 ml of arsenic standard solution (1 ppm).

Lead (2.3.15). Prepare two solutions as follows. For solution (a) add 5 ml of *6 M acetic acid* to 12 g of the syrup. For solution (b) add 5 ml of *6 M acetic acid* and 2 ml of *lead standard solution (10 ppm Pb)* to 2.0 g of the syrup. Make solution (a) and (b) alkaline with *5 M ammonia*, if necessary, and to each add 1 ml of *potassium cyanide solution PbT*. The solutions should not be more than faintly opalescent. If the colours of the solutions differ, equalise by the addition of a few drops of a highly diluted solution of burnt sugar or other non-reactive substance. Dilute each solution to 50 ml with *water*, add 0.1 ml of a 10 per cent w/v solution of *sodium sulphide* to each and mix thoroughly. When viewed against a white background, the colour produced in solution (a) is not more intense than that produced in solution (b) (2 ppm).

Refractive index (2.4.27). 1.4608 to 1.4630.

Sulphur dioxide (2.3.40). Not more than 70 ppm.

Weight per ml (2.4.29). 1.338 to 1.344 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Dilute the syrup so that the volume of the diluted solution required in the following method is between 15 to 50 ml. Add 10.0 ml of *cupri-tartaric solution* to a 300-ml conical flask, add from a burette 15 ml of the diluted solution, heat to boiling over wire gauze covered with insulating material and continue adding the diluted solution in quantities of about 5 ml at 15-second intervals until the colour of the mixture indicates that the reduction appears to be almost complete. Boil for 2 minutes, add 0.2 ml of a 1 per cent w/v solution of *methylene blue* and continue the titration until the blue colour is discharged. Repeat the operation, but, before heating, add almost the full quantity of the diluted solution required to reduce all the copper and then boil moderately for 2 minutes. Without removing the flask from either the gauze or the flame during the remainder of the titration, add 0.2 ml of the *methylene blue* solution and continue the titration so that it is just complete in a total boiling time of exactly 3 minutes, the end point is indicated by the disappearance of the blue colour, the solution becoming orange.

Calculate the content of reducing sugar expressed as invert sugar in 100 ml of the diluted solution from the table.

Quantity of prepared solution required ml	Invert sugar factor*	Quantity of invert sugar per 100 ml mg
15	50.5	336.0
16	50.6	316.0
17	50.7	298.0
18	50.8	282.0
19	50.8	267.0
20	50.9	254.5
21	51.0	242.9
22	51.0	231.8
23	51.1	222.2
24	51.2	213.3
25	51.2	204.8
26	51.3	197.4
27	51.4	190.4
28	51.4	183.7
29	51.5	177.6
30	51.5	171.7
31	51.6	166.3
32	51.6	161.2
33	51.7	156.6
34	51.7	152.2
35	51.8	147.9
36	51.8	143.9
37	51.9	140.2
38	51.9	136.6
39	52.0	133.3
40	52.0	130.1
41	52.1	127.1
42	52.1	124.2
43	52.2	121.4
44	52.2	118.7
45	52.3	116.1
46	52.3	113.7
47	52.4	111.4
48	52.4	109.2
49	52.5	107.1
50	52.5	105.1

* mg of invert sugar corresponding to 10.0 ml of *cupri-tartaric solution*.

Storage. Store at a temperature between 35° to 45°.

Iodine

I_2 Mol. Wt. 253.8

Iodine contains not less than 99.5 per cent and not more than 100.5 per cent of I_2.

Category. Antiseptic; used in the treatment of thyroid deficiency.

Description. Greyish violet brittle plates or small crystals with a metallic sheen. It volatalises slowly at room temperature.

Identification

A. When heated gently it gives violet vapours which condense forming a bluish-black crystalline sublimate.

B. A saturated solution yields a blue colour in the presence of *starch solution* which disappears when the solution is heated and reappears when it is cooled.

Tests

Bromides and chlorides. Not more than 250 ppm, determined by the following method. Triturate 3.0 g with 20 ml of *water*, filter, wash the filter, dilute the filtrate to 30 ml with *water* and add 1 g of *zinc powder*. When the solution is decolorised, filter and wash the filter with sufficient *water* to produce 40 ml of filtrate. To 10 ml of the solution add 3 ml of *10 M ammonia* and 6 ml of *silver nitrate solution*, filter, wash the filter with *water* and dilute to 20 ml with *water*. To 10 ml of the filtrate add 1.5 ml of *nitric acid*. After 1 minute any opalescence produced is not more intense than that produced in a solution prepared at the same time by mixing 10.75 ml of *water*, 0.25 ml of *0.01 M hydrochloric acid*, 0.2 ml of *2 M nitric acid* and 0.3 ml of *silver nitrate solution*.

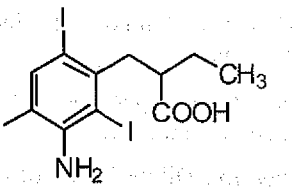
Non-volatile matter. Not more than 0.1 per cent, determined by heating 1.0 g in a porcelain dish on a water-bath until the iodine has volatilised and drying the residue at 105°.

Assay. Weigh 0.2 g, transfer to a flask containing 1 g of *potassium iodide* and 2 ml of *water*, add 1 ml of *2 M acetic acid*, dissolve completely and add 50 ml of *water*. Titrate with *0.1 M sodium thiosulphate* using *starch solution* as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.01269 g of I.

Storage. Store in ground-glass-stoppered containers or in earthenware containers with waxed bungs.

Iopanoic Acid



$C_{11}H_{12}I_3NO_2$ Mol. Wt. 570.9

Iopanoic Acid is (*RS*)-2-(3-amino-2,4,6-tri-iodobenzyl)butanoic acid.

Iopanoic Acid contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{11}H_{12}I_3NO_2$, calculated on the dried basis.

Category. Antihyperthyroidism.

Description. A white or yellowish-white powder.

Identification

Tests B and C may be omitted if tests A is carried out. Tests A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *iopanoic acid IPRS* or with the reference spectrum of iopanoic acid.

B. Spray the plate obtained in the test for Related substances with a 0.1 per cent w/v solution of *4-dimethylamino-cinnamaldehyde* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of *ethanol (95 per cent)*. The principal spot in the chromatogram obtained with test solution (b) corresponds to the spot in the chromatogram obtained with reference solution (a).

C. Heat 50 mg carefully in a small porcelain dish over a flame; violet vapour is evolved.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *1 M sodium hydroxide* is clear (2.4.1) and not more intensely coloured than reference solution YS3 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *concentrated ammonia*, 20 volumes of *methanol*, 20 volumes of *toluene* and 50 volumes of *dioxan*.

Solvent mixture. 3 volumes of *ammonia* and 97 volumes of *methanol*.

Test solution (a). Dissolve 1.0 g of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture.

Reference solution (a). A 1.0 per cent w/v solution of *iopanoic acid IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (b) to 50.0 ml with the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Chlorides (2.3.12). To 1.38 g, add 10 ml of *nitric acid* and 5 ml of *water*, shake for 5 minutes and filter. The solution complies with the limit test for chlorides (180 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

Assay. To 0.15 g in a 250 ml round-bottomed flask, add 5 ml of *strong sodium hydroxide solution*, 20 ml of *water*, 1 g of *zinc powder* and a few glass beads. Boil under a reflux condenser for 60 minutes. Allow to cool and rinse the condenser with 20 ml of *water*, adding the rinsings to the flask. Filter through a sintered-glass filter and wash the filter with several quantities of *water*. Collect the filtrate and washings. Add 40 ml of *dilute sulphuric acid* and titrate immediately with *0.1 M silver nitrate*. Determine the end-point potentiometrically (2.4.25), using a suitable electrode system such as silver-mercurous sulphate.

1 ml of *0.1 M silver nitrate* is equivalent to 0.01903 g of $C_{11}H_{12}I_3NO_2$.

Storage. Store protected from light.

Iopanoic Acid Tablets

Iopanoic Acid Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of iopanoic acid, $C_{11}H_{12}I_3NO_2$.

Usual strength. 500 mg.

Identification

A. Shake a quantity of the powdered tablets containing 30 mg of Iopanoic Acid with 10 ml of *ethanol (95 per cent)*, filter, evaporate the filtrate to dryness and dry the residue at 105°. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *iopanoic acid IPRS* or with the reference spectrum of iopanoic acid.

B. Spray the chromatograms obtained in the test for Related substances with a 0.1 per cent w/v solution of *dimethylamino-cinnamaldehyde* in a mixture of 1 volume of *hydrochloric acid* and 99 volumes of *ethanol (95 per cent)*. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Chlorides (2.3.12). To a quantity of the powdered tablets containing 2.3 g of Iopanoic Acid, add just sufficient *0.2 M sodium hydroxide* to dissolve the iopanoic acid, dilute to 15 ml with *water*, add sufficient *2 M nitric acid* dropwise to ensure complete precipitation of the iodinated acid and add 3 ml in excess. Filter, wash the precipitate with 5 ml of *water* and combine the solutions and dilute to 25 ml with *water*. 15 ml of the solution complies with the limit test for chlorides (180 ppm).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *13.5 M ammonia*, 20 volumes of *methanol*, 20 volumes of *toluene* and 50 volumes of *1,4-dioxan*.

Solvent mixture. 3 volumes of *10 M ammonia* and 97 volumes of *methanol*.

Test solution (a). Extract a quantity of the powdered tablets containing 1.0 g of Iopanoic Acid with five 10-ml quantities of *ethanol (95 per cent)*, filter, evaporate the combined filtrates to dryness using a rotary evaporator and dissolve the residue in 10 ml of the solvent mixture.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of test solution (a) to 500.0 ml with the solvent mixture.

Reference solution (b). A 1.0 per cent w/v solution of *iopanoic acid IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the plate to rise 10 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent).

Other tests. Comply with the tests stated under Tablets.

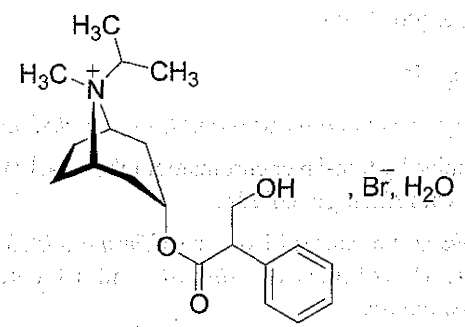
Assay. Weigh and powder 20 tablets. Triturate a quantity of the powder containing 0.4 g of Iopanoic Acid with five 10-ml

quantities of *ethanol (95 per cent)*, decanting and filtering each extract through the same filter. Evaporate the combined filtrates almost to dryness on a water-bath, cool, add 20 ml of *water*, 12 ml of *5 M sodium hydroxide* and 1 g of *zinc powder* and boil under a reflux condenser for 30 minutes. Cool, rinse the condenser with 30 ml of *water*, filter through absorbent cotton and wash the flask and filter with two 20-ml quantities of *water*. To the combined filtrate and washings, add 80 ml of *hydrochloric acid*, cool and titrate with *0.05 M potassium iodate* until the dark brown solution becomes light brown. Add 5 ml of *chloroform* and continue the titration, shaking well after each addition, until the chloroform becomes colourless.

1 ml of *0.05 M potassium iodate* is equivalent to 0.01903 g of $C_{11}H_{12}I_3NO_2$.

Storage. Store protected from light.

Ipratropium Bromide



$C_{20}H_{30}BrNO_3,H_2O$ Mol. Wt. 430.4

Ipratropium Bromide is 3α-Hydroxy-8-isopropyl-1α*H*,5α*H*-tropanium bromide (*RS*)-tropate monohydrate.

Ipratropium Bromide contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{20}H_{30}BrNO_3$, calculated on the anhydrous basis.

Category. Anticholinergic; bronchodilator.

Description. A white or almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ipratropium bromide IPRS* or with the reference spectrum of ipratropium bromide.

B. It gives reaction (A) of bromides (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

Impurity A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 1 volume of *formic acid*, 3 volumes of *water*, 18 volumes of *ethanol (95 per cent)* and 18 volumes of *dichloromethane*.

Test solution. Dissolve 20 mg of the substance under examination in *methanol* and dilute to 1.0 ml with *methanol*.

Reference solution (a). Dissolve 20 mg of *ipratropium bromide IPRS* in *methanol* and dilute to 1.0 ml with *methanol*.

Reference solution (b). Dissolve 20 mg of *methylatropine bromide IPRS* in 1.0 ml of reference solution (a).

Reference solution (c). Dissolve 5 mg of *ipratropium impurity A IPRS {(1R,3r,5S,8r)-3-hydroxy-8-methyl-8-(1-methylethyl)-8-azoniabicyclo [3.2.1]octane IPRS}* in 100.0 ml of *methanol*. Dilute 2.0 ml of the solution to 5.0 ml with *methanol*.

Apply to the plate 1 µl of each solution. After development, dry the plate at 60° for 15 minutes, spray with *potassium iodobismuthate solution*, dry briefly in a current of air and spray with a 5.0 per cent w/v solution of *sodium nitrite*. The chromatogram obtained with reference solution (b) shows two clearly separated principal spots. In the chromatogram obtained with the test solution any spot corresponding to ipratropium impurity A is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.001 per cent w/v solution of *ipratropium bromide IPRS* in the mobile phase.

Reference solution (b). Dissolve 5 mg each of *ipratropium bromide IPRS* and *ipratropium impurity B IPRS (1R,3r,5S,8s)-3-[[(2RS)-3-hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane IPRS)* in 1 ml of *methanol* and dilute to 25.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 87 volumes of buffer solution prepared by dissolving 1.24 g of *sodium dihydrogen phosphate* and 0.17 g of *tetrapropylammonium chloride* in 87 ml of *water* and adjusted to pH 5.5 with 18 per cent w/v solution of *disodium hydrogen phosphate*, and 13 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Bromide ion	0.15	---
Ipratropium impurity C[1]	0.7	0.3
Ipratropium (Retention time: about 4.9 minutes)	1.0	---
Ipratropium impurity B[2]	1.2	---
Ipratropium impurity D[3]	1.8	0.2
Ipratropium impurity E[4]	2.3	---
Ipratropium impurity F[5]	5.1	0.5

[1](2*RS*)-3-hydroxy-2-phenylpropanoic acid (dl-tropic acid),

[2](1*R*,3*r*,5*S*,8*s*)-3-[[(2*RS*)-3-hydroxy-2-phenylpropanoyl]oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane,

[3]2-phenylpropenoic acid,

[4](1*R*,3*r*,5*S*)-8-(1-methylethyl)-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-3-hydroxy-2-phenylpropanoate,

[5](1*R*,3*r*,5*S*,8*r*)-8-methyl-8-(1-methylethyl)-3-[(2-phenylpropenoyl) oxy]-8-azoniabicyclo[3.2.1]octane.

Inject reference solution (b). The test is not valid unless the resolution between ipratropium and ipratropium impurity B is not less than 3.0 and the tailing factor is not more than 2.5 for the principal peak.

Inject reference solution (a) and the test solution. Run the chromatogram 6 times the retention times of the ipratropium peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to ipratropium impurity D is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent), the area of any peak corresponding to ipratropium impurity B and ipratropium impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent). Ignore any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent), disregard the peak due to the bromide ion.

Water (2.3.43). 3.9 per cent to 4.4 per cent, determined on 0.5 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh 0.35 g of the substance under examination, dissolve in 50 ml of *water* and add 3 ml of *dilute nitric acid*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.04124 g of $C_{20}H_{30}BrNO_3$.

Storage. Store protected from light and moisture.

Ipratropium Powder for Inhalation

Ipratropium Bromide Powder for Inhalation

Ipratropium Powder for Inhalation consists of hard gelatin capsules containing Ipratropium Bromide in microfine powder either alone or admixed with glucose in a pre-metered unit for use in a suitable powder inhaler.

The powder for inhalation complies with the requirements stated under Inhalation Preparation and the contents of the capsules comply with the following requirements.

Ipratropium Powder for Inhalation contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ipratropium bromide, $C_{20}H_{30}NO_3Br,H_2O$ per unit dose.

Usual strength. 40 µg.

Identification

Test A may be omitted if test B and C are carried out.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *formic acid*, 3 volumes of *water*, 18 volumes of *ethanol* and 18 volumes of *dichloromethane*.

Test solution. Shake vigorously a quantity of the contents of the capsules containing about 1.84 mg of Ipratropium Bromide with 1 ml of *methanol* for 2 minutes, centrifuge and use the supernatant.

Reference solution (a). A 0.1 per cent w/v solution of *ipratropium bromide IPRS* in a saturated solution of *glucose*.

Reference solution (b). A 0.005 per cent w/v solution each of *(1R,3r,5S,8s)-3-[[(2RS)-3-hydroxy-2-phenylpropanoyl] oxy]-8-methyl-8-(1-methylethyl)-8- azoniabicyclo[3.2.1] octane IPRS (ipratropium bromide impurity B)* and *ipratropium bromide IPRS* in *0.001M hydrochloric acid*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 5 cm. Dry the plate for 15 minutes at 60°, spray with *potassium iodobismuthate solution*, dry in a current of air and spray with a 5.0 per cent w/v solution of *sodium nitrite*. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

B. Disperse a quantity of the contents of the capsules containing about 0.3 mg of bromide ion in 0.5 ml of *water*, add

75 mg of *lead dioxide* and 0.25 ml of *acetic acid*, shake and allow to stand for 5 minutes. Add one drop to a strip of filter paper previously impregnated with *decolorised fuchsin solution*, a violet colour develops.

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 1 volume of *formic acid*, 3 volumes of *water*, 18 volumes of *ethanol* and 18 volumes of *dichloromethane*.

Test solution. Shake a quantity of the contents of the capsules containing about 0.1 g of Ipratropium Bromide with 1 ml of *methanol* for 2 minutes, centrifuge and use the supernatant.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with a saturated solution of *glucose*.

Reference solution (b). A 0.0005 per cent w/v solution of *tropic acid* in a saturated solution of *glucose*.

Apply to the plate 10 µl of each solution. After development, dry the plate at 60° for 15 minutes, spray with *potassium iodobismuthate solution*, dry briefly in a current of air and spray with a 5.0 per cent w/v solution of *sodium nitrite*. In the chromatogram obtained with the test solution any spot corresponding to tropic acid is not more intense than the spot in the chromatogram obtained with reference solution (a) 0.5 per cent); any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

B. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the content of capsules containing about 20 mg of Ipratropium Bromide with mobile phase and dilute to 100.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b). A 0.005 per cent w/v solution each of *(1R,3r,5S,8s)-3-[[(2RS)-3-hydroxy-2-phenylpropanoyl] oxy]-8-methyl-8-(1-methylethyl)-8- azoniabicyclo[3.2.1] octane IPRS* (*ipratropium bromide impurity B IPRS*) and *ipratropium bromide IPRS* in *0.001M hydrochloric acid*.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Lichrospher 60 RP-select B),
- mobile phase: a mixture of 3 volumes of *acetonitrile* and 25 volumes of a 0.1 per cent w/v solution of *sodium methanesulphonate* in *0.05 M orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 1.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Inhalation Preparations (Powders for Inhalation).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the contents of the capsules with sufficient *0.01 M hydrochloric acid* to produce a solution containing 0.004 per cent w/v of Ipratropium Bromide.

Reference solution (a). A 0.004 per cent w/v solution of *ipratropium bromide IPRS* in *0.01 M hydrochloric acid*.

Reference solution (b). A 0.005 per cent w/v solution each of *(1R,3r,5S,8s)-3-[[(2RS)-3-hydroxy-2-phenylpropanoyl] oxy]-8-methyl-8-(1-methylethyl)-8- azoniabicyclo[3.2.1] octane IPRS* (*ipratropium bromide impurity B IPRS*) and *ipratropium bromide IPRS* in *0.001M hydrochloric acid*.

Chromatographic system

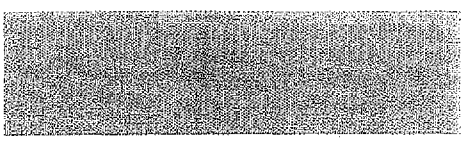
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Lichrospher RP8 select B),
- mobile phase: a mixture of 27 volumes of *acetonitrile* and 100 volumes of a 0.2 per cent w/v solution of *sodium heptanesulphonate monohydrate* in *water*, adjusted to pH 3.2 with *0.05 M orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 1.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{20}H_{30}NO_3Br,H_2O$ in the powder for inhalation.

Labelling. The label states the quantity of Ipratropium Bromide contained in each capsule.



Ipratropium Inhalation

Ipratropium Bromide Inhalation

Ipratropium Inhalation is a solution or suspension of Ipratropium Bromide in a suitable liquid in a suitable pressurised container.

The pressurised inhalation complies with the requirements stated under Inhalation Preparation and with the following requirements.

Ipratropium Inhalation delivers not less than 85.0 per cent and not more than 115.0 per cent of the stated amount of ipratropium bromide, $C_{20}H_{30}NO_3Br,H_2O$, per inhalation by actuation of the valve.

Usual strengths. 20 µg per metered dose; 40 µg per metered dose.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with reference solution (a) corresponds to that in the chromatogram obtained with reference solution (e).

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. 5 volumes of *water*, 8 volumes of *anhydrous formic acid*, 28 volumes of *methanol* and 70 volumes of *dichloromethane*.

Test solution. Punch a small hole in the ferrule of each of three cooled containers, allow the propellant to evaporate for about 1 minute and transfer the contents of the containers, through the punched holes, to a beaker. Stir, using a magnetic stirrer, for about 10 minutes, add 3.5 ml of *0.01M hydrochloric acid* and continue stirring for about 1 hour, until the propellant has completely evaporated, filter, add 10 ml of *chloroform* to the filtrate and shake vigorously for 1 minute. Allow the phases to separate and use the upper layer.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with *0.01 M hydrochloric acid*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 4.0 ml with *0.01 M hydrochloric acid*.

Reference solution (c). Dilute 2.0 ml of reference solution (b) to 5.0 ml with *0.01 M hydrochloric acid*.

Reference solution (d). A 0.008 per cent w/v solution of *8s-isopropyl-3b-hydroxytropanium bromide IPRS (ipratropium impurity A IPRS)* in *0.01 M hydrochloric acid*.

Reference solution (e). A 0.008 per cent w/v solution of *ipratropium bromide IPRS* in *0.01 M hydrochloric acid*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 6 cm. Dry the plate in a current of warm air for about 30 minutes. Spray the plate with a mixture of 1 volume of *potassium iodobismuthate solution*, 2 volumes of *glacial acetic acid* and 10 volumes of *water*, allow to dry, spray with a 5 per cent w/v solution of *sodium nitrite* and immediately examine the plate. In the chromatogram obtained with the test solution, any spot corresponding to *8s*-isopropyl-3b-hydroxytropanium bromide is not more intense than the spot in the chromatogram obtained with reference solution (a) (2.0 per cent). Any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

Other tests. Comply with the tests stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *0.001 M hydrochloric acid* and *methanol*.

Test solution. Prepare using the solvent mixture as described under the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Reference solution. A 0.0004 per cent w/v solution of *ipratropium bromide IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octylsilane bonded to porous silica (Such as Lichrospher RP8 select B) (5 µm),
- mobile phase: a mixture of 34.5 volumes of *acetonitrile* and 75 volumes of 0.012 M *sodium heptanesulphonate*, adjusted to pH 3.2 with *0.05 M orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

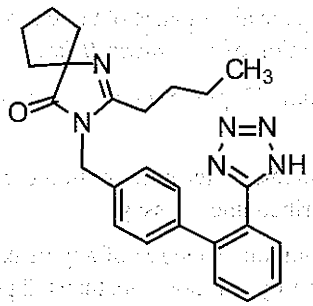
Inject the reference solution. The test is not valid unless the tailing factor is not less than 3.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{30}BrNO_3,H_2O$ delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of $C_{20}H_{30}BrNO_3,H_2O$ delivered per actuation of the valve meets the requirements.

Irbesartan



$C_{25}H_{28}N_6O$ Mol. Wt. 428.5

Irbesartan is 2-butyl-3-{4-[2-(1*H*-tetrazol-5-yl)phenyl]benzyl}-1,3-diazaspiro[4.4]non-1-en-4-one.

Irbesartan contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{25}H_{28}N_6O$, calculated on the anhydrous basis.

Category. Antihypertensive.

Production

As Nitrosamines are classified as probable human carcinogens, their presence in irbesartan should be avoided or limited as much as possible. For this reason, manufacturers of irbesartan for human use are expected to perform an assessment of the risk of *N*-nitrosamine formation and contamination during their manufacturing process; if this assessment identifies a potential risk, the manufacturing process should be modified to minimise contamination and a control strategy implemented to detect and control *N*-nitrosamine impurities in irbesartan. The general chapter 5.11. Nitrosamine Impurities is available to assist manufacturers.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *irbesartan IPRS* or with the reference spectrum of irbesartan.

Tests

Appearance of solution. A 5.0 per cent w/v solution in 1 volume of *2 M sodium hydroxide* and 9 volumes of *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BS7 (2.4.1).

Impurity B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in the mobile phase, dilute to 5.0 ml with the mobile phase.

Reference solution. A 0.00003 per cent w/v solution of *sodium azide* (sodium salt of irbesartan impurity B) in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with strongly basic anion-exchange resin (8.5 µm),
- mobile phase: a 0.42 per cent w/v solution of *sodium hydroxide* in *carbon dioxide-free water*,
- flow rate: 1 ml per minute,
- conductivity detector with a sensitivity of 3 µS,
- injection volume: 200 µl.

Inject the reference solution. The test is not valid unless, the signal to noise ratio for the principal peak is not less than 10.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to irbesartan impurity B is not more than the area of the principal peak in the chromatogram obtained with the reference solution (10 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *methanol*, dilute to 50.0 ml with *methanol*.

Reference *solution (a)*. Dilute 1.0 ml of the test solution to 20.0 ml with *methanol*. Dilute 1.0 ml of the solution to 50.0 ml with *methanol*.

Reference solution (b). A solution containing 0.005 per cent w/v each of *irbesartan IPRS* and *irbesartan impurity A IPRS (1-(pentanoylamino)-N-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]cyclopentane-carboxamide IPRS)* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 67 volumes of buffer solution prepared by diluting 5.5 ml of *orthophosphoric acid* in 950 ml of *water*, adjusted to pH 3.2 with *triethylamine* and 33 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl

The relative retention time with reference to irbesartan (retention time: about 23 minutes) for irbesartan impurity A is about 0.7.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to irbesartan impurity A and irbesartan is not less than 3.0.

Inject reference solution (a) and the test solution. Run the chromatogram 1.4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding irbesartan impurity A is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the areas of any other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13.). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Dissolve 0.3 g in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04285 g of $C_{25}H_{28}N_6O$.

Irbesartan Tablets

Irbesartan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of irbesartan, $C_{25}H_{28}N_6O$.

Usual strengths. 150 mg; 300 mg.

Identification

A. Transfer one tablet into a suitable vial, add 10 ml of *methanol*, sonicate for 10 minutes, filter and evaporate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *irbesartan IPRS* or with the reference spectrum of irbesartan.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 1000 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 244 nm (2.4.7). Calculate the content of $C_{25}H_{28}N_6O$ in the medium from the absorbance obtained from a solution of known concentration of *irbesartan IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{25}H_{28}N_6O$.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay.

Inject the test solution. The area of any peak corresponding to irbesartan impurity A is not more than 0.2 per cent, the area of any other secondary peak is not more than 0.2 per cent and the sum of areas of all the secondary peaks is not more than 0.5 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 Tablets. Disperse a quantity of the powder containing 15 mg of irbesartan in 75 ml of *methanol* with the aid of ultrasound and dilute to 100.0 ml with *methanol*.

Reference solution (a). A 0.015 per cent w/v solution of *irbesartan IPRS* in *methanol*.

Reference solution (b). A solution containing 0.01 per cent w/v of *irbesartan IPRS* and *irbesartan impurity A IPRS (1-pentanoylamino-cyclopentanecarboxylic acid [2'-(1H-tetrazol-5-yl)-byphenyl-4-ylmethyl]-amide IPRS)* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by diluting 5.5 ml of *orthophosphoric acid* in 950 ml of *water*, adjusted to pH 3.0 with *triethylamine* and dilute to 1000 ml with *water* and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 15 µl.

Inject reference solution (b). The test is not valid unless the resolution between the irbesartan and irbesartan impurity A is not less than 2.0

Inject reference solution (a) and the test solution.

Calculate the content of $C_{25}H_{28}N_6O$ in the tablets.

Storage. Store protected from moisture.

Irbesartan and Hydrochlorothiazide Tablets

Irbesartan and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of irbesartan, $C_{25}H_{28}N_6O$ and hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

Usual strength. 150 mg of irbesartan and 12.5 mg of hydrochlorothiazide.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2)

Apparatus No. 2 (Paddle),

Medium. 1000 ml of *0.1 M hydrochloric acid*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Dissolve a quantity of *irbesartan IPRS* and *hydrochlorothiazide IPRS* in sufficient *methanol* and dilute with dissolution medium to obtain a solution having a known concentration similar to the expected concentration of test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile group chemically bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 45 volumes of a buffer solution prepared by dissolving 1.36 g of *monobasic potassium phosphate* in 1000 ml of *water*, 35 volumes of *methanol* and 20 volumes of *acetonitrile*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 25 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{28}N_6O$ and $C_7H_8ClN_3O_4S_2$ in the tablet.

Q. Not less than 80 per cent of the stated amount of $C_{25}H_{28}N_6O$ and $C_7H_8ClN_3O_4S_2$.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Name	Relative retention time
Benzothiadiazine impurity A[1]	0.15
Hydrochlorothiazide	0.18
Irbesartan impurity A[2]	0.86
Irbesartan	1.0

[1]4-amino-6-chloro-1,3-benzenedisulfonamide,

[2]1-pentanoylamino-cyclopentanecarboxylic acid [2'-(*1H*-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amide.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to irbesartan impurity A is not more than 0.3 times the area of the peak due to irbesartan in the chromatogram obtained with reference solution (c) (0.3 per cent), the area of any peak corresponding to benzothiadiazine impurity A, multiplied by correction factor of 0.77, is not more than 0.94 times the area of the peak due to hydrochlorothiazide in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any other secondary peak is not more than 0.2 times the area of the peak due to irbesartan in the chromatogram obtained with reference solution (c) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 1.5 times the area of the peak due to irbesartan in the chromatogram obtained with reference solution (c) (1.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *methanol* and 30 volumes of acidified *water* previously adjusted to pH 2.0 with *orthophosphoric acid* or *sodium hydroxide*.

Test solution. Disperse a quantity of powdered tablets containing 22.5 mg of Irbesartan in 100.0 ml of the solvent mixture.

Reference solution (a). A solution containing 0.024 per cent w/v of *irbesartan IPRS* and 0.002 per cent w/v of *hydrochlorothiazide IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.005 per cent w/v of *irbesartan IPRS*, 0.0005 per cent of wv of *hydrochlorothiazide IPRS*, 0.0001 per cent w/v of *irbesartan impurity A IPRS* and 0.0003 per cent w/v of *benzothiadiazine impurity A IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 67 volumes of the buffer solution, prepared by dissolving 1.36 g of *monobasic potassium phosphate* in 900 ml of *water*, add 2 ml of *triethylamine*, adjusted to pH 3.0 with *orthophosphoric acid*, dilute to 1000 ml with *water*, 13 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

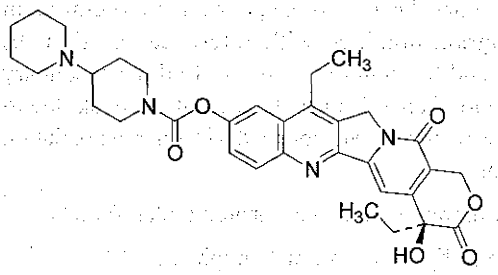
Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to irbesartan and irbesart impurity A is not less than 1.7, between the peaks corresponding to hydrochlorothiazide and benzothiadiazine impurity A is not less than 1.7. The relative standard deviation for replicate injections for irbesartan and hydrochlorothiazide peak is not more than 1.5 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{25}H_{28}N_6O$ and $C_7H_8ClN_3O_4S_2$ in the tablets.

Storage. Store protected from moisture.

Irinotecan Hydrochloride Trihydrate



$C_{33}H_{38}N_4O_6.HCl,3H_2O$ Mol. Wt. 677.2

Irinotecan Hydrochloride Trihydrate is (4*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1*H*-pyrano [3',4' :6,7]indolizino[1 ,2-*b*]quinolin-9-yl [1 ,4'-bipiperidine]- 1'-carboxylate hydrochloride trihydrate.

Irinotecan Hydrochloride Trihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{33}H_{38}N_4O_6$, HCl, calculated on the anhydrous basis.

Category. Anticancer

Description. A pale yellow to yellow crystalline powder.

CAUTION — Irinotecan Hydrochloride Trihydrate is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *irinotecan hydrochloride trihydrate IPRS* or with the reference spectrum of irinotecan hydrochloride trihydrate.

B. In the Enantiomeric purity test, the principal peak in the chromatogram obtained with the test solution corresponds to the irinotecan (S-enantiomer) peak in the chromatogram obtained with reference solution (b).

C. A 0.2 per cent w/v solution gives reaction (A) of chlorides (2.3.1).

Tests

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *ethanol* and 0.1 volume of *diethylamine*.

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.01 per cent w/v, each of, *irinotecan hydrochloride trihydrate IPRS* and *irinotecan related compound D IPRS* in the solvent mixture.

Reference solution (b). A 0.1 per cent w/v solution of *irinotecan hydrochloride trihydrate IPRS* in the solvent mixture.

Reference solution (c). A 0.00015 per cent w/v solution of *irinotecan related compound D IPRS* in the solvent mixture.

Reference solution (d). Dilute 5.0 ml of reference solution (c) to 15.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with cellulose tris-3,5-dimethylphenylcarbamate coated porous silica (10 µm) (Such as chiralcel OD-H),
- mobile phase: a mixture of 25 volumes of *hexane*, 25 volumes of *ethanol*, 0.1 volume of *diethylamine*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 370 nm,
- injection volume: 20 µl.

Inject reference solution (a), (b), (c) and (d). The test is not valid unless the resolution between the peaks due to irinotecan related compound D and irinotecan hydrochloride trihydrate is not less than 2.5 in the chromatogram obtained with reference solution (a), the relative standard deviation for replicate injections is not more than 5.0 in the chromatogram obtained with reference solution (c) and the irinotecan hydrochloride related compound D peak should be visible in the chromatogram obtained with reference solution (d).

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution the area of the peak due to irinotecan related compound D (R-enantiomer) is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.15 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

A. *For material labelled as produced by a synthetic process—*

Buffer solution. Dissolve 2.8 g of *monobasic sodium phosphate monohydrate* and 1.8 g of *octanesulphonic acid sodium salt monohydrate* in 1000 ml of *water*.

Solvent mixture. 59 volumes of buffer solution, 24 volumes of *methanol* and 17 volumes of *acetonitrile*, adjusted to pH *3.65* with *dilute hydrochloric acid*.

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution (a). A solution containing 0.0001 per cent w/v, each of, *irinotecan related compound B IPRS* and *irinotecan related compound C IPRS* in *methanol*.

Reference solution (b). A 0.0002 per cent w/v solution of *irinotecan hydrochloride trihydrate IPRS* in the solvent mixture.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 20.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 59 volumes of buffer solution, 24 volumes of *methanol* and 17 volumes of *acetonitrile*,
- a gradient programme using the conditions below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 255 nm,
- injection volume: 15 µl.

Name	Relative retention time
Irinotecan related compound B[1]	0.55
Irinotecan related compound C[2]	0.6
Irinotecan hydrochloride	1.0

[1] *(S)*-4,11-Diethyl-4,9-dihydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione,

[2] 11-Ethyl-4-hydroxy-4-methyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-9-yl(1,4'-bipiperidine)-1'-carboxylate hydrochloride.

Inject reference solution (a), (b) and (c). The test is not valid unless the resolution between the peaks due to irinotecan related compound B and irinotecan related compound C is not less than 1.1 in the chromatogram obtained with reference solution (a), the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (b) and the signal-to-noise ratio is not less than 10 in the chromatogram obtained with reference solution (c).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to irinotecan related compound B is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any peak corresponding to irinotecan related compound C is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

B. *For material labelled as produced by a semi-synthetic process—*

Solvent mixture. 10 volumes of *acetonitrile*, 10 volumes of *methanol* and 20 volumes of mobile phase A

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution (a). A 0.0001 per cent w/v solution of *irinotecan hydrochloride trihydrate IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v, each of, *irinotecan hydrochloride trihydrate IPRS* and *irinotecan related compound A IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *dilute orthophosphoric acid*,
  B. a mixture 60 volumes of *acetonitrile* and 40 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
40	30	70
45	30	70
50	80	20
55	80	20

Name	Relative retention time	Correction factor
7-Desethyl Irionotecan[1]	0.82	1.3
Irinotecan	1.00	—
Irinotecan related compound A[2]	1.15	0.71
11-Ethyl irinotecan[3]	1.27	1.59
Camptothecin[4]	1.35	0.71
Irinotecan related compound B[5]	1.50	0.77
7-Ethylcamptothecin[6]	1.76	0.83
7,11-Diethyl-10-hydroxy camptothecin[7]	2.05	1.54

[1]*(S)*-4-Ethyl-4-hydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*] quinoline-3,14(4H,12H)-dione-9-yl (1,4'-bipiperidine)- 1'-carboxylate,

[2]*(S)*-4-Ethyl-4,9-hydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*] quinoline-3,14(4H,12H)-dione,

[3]*(S)*-4,8,11-Triethyl-4-hydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*] quinoline-3,14(4H,12H)-dione-9-yl (1,4'-bipiperidine)- 1'-carboxylate,

[4]*(S)*-4-Ethyl-4-hydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*] quinoline-3,14(4H,12H)-dione,

[5]*(S)*-4,11-Diethyl-4,9-dihydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-3,14(4H,12H)-dione,

[6]*(S)*-4,11-Diethyl-4-hydroxy-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*] quinoline-3,14(4H,12H)-dione,

[7]*(S)*-4,8,11-Triethyl-4,9-dihydroxy-1*H*-pyrano[3',4':6,7]indolizino [1,2-*b*]quinoline-3,14(4H,12H)-dione.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to irinotecan and irinotecan related compound A is not less than 3.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 7-desethyl irinotecan, irinotecan related compound A, 11-ethyl irinotecan, camptothecin, irinotecan related compound B, 7-ethylcamptothecin, 7,11-diethyl-10-hydroxy camptothecin, each of, is not more than 1.5 times the area of the peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than the 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1 .0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 7.0 per cent to 9.0 per cent, determined on 0.1 g.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 10^2 CFU per g. 1 g is free from *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. 10 g is free from *Salmonella* and *Shigella*.

Bacterial endotoxins (2.2.3). Not more than 0.275 Endotoxin Unit per mg of irinotecan hydrochloride trihydrate.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances A with the following modifications.

Reference solution. A 0.1 per cent w/v solution of *irinotecan hydrochloride trihydrate IPRS* in the solvent mixture.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{33}H_{38}N_4O_6,HCl$.

Storage. Store protected from light, at a temperature not exceeding 25°.

Irinotecan Injection

Irinotecan Hydrochloride Injection

Irinotecan Injection is a sterile solution of Irinotecan Hydrochloride Trihydrate in *water*.

Irinotecan Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of irinotecan hydrochloride trihydrate, $C_{33}H_{38}N_4O_6,HCl,3H_2O$.

Usual strength. 20 mg per ml.

Description. A light yellow coloured solution, free from visible particles.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. It gives the reaction of chlorides (2.3.1).

Tests

Appearance of solution. Prepare a solution equivalent to 1.0 per cent of the substance in *water*. The solution is clear or not more intensely coloured than the reference solution *GYS3* (2.4.1).

pH (2.4.24). 3.0 to 3.8.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a measured volume containing 40 mg of Irinotecan Hydrochloride Trihydrate to 100 ml with mobile phase.

Reference solution (a). A 0.04 per cent w/v solution of *irinotecan hydrochloride trihydrate IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with mobile phase.

Reference solution (c). Dissolve 20 mg of *irinotecan hydrochloride trihydrate IPRS* in 10 ml of 0.04 per cent w/v solution of *7-ethyl-10-hydroxycamptothecin IPRS* in *N,N,dimethyl-formamide* and dilute to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 72 volumes of *buffer solution* prepared by dissolving 1.1 g of *1-heptane sulphonic acid sodium monohydrate* and 7.1 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 28 volumes of *acetonitrile*, filter.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject reference solution (c). The test is not valid unless the resolution between 7-ethyl-10-hydroxycamptothecin and irinotecan hydrochloride trihydrate is not less than 3.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with the reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 1.2 times the area of the peak in the chromatogram obtained with the reference solution (b) (1.2 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.556 Endotoxin Unit per mg of irinotecan hydrochloride trihydrate.

Sterility (2.2.11). Complies with the test for sterility.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Measured volume of the injection containing 40 mg of *irinotecan hydrochloride trihydrate*, diluted to 100.0 ml with mobile phase.

Reference solution. A 0.04 per cent w/v solution of *irinotecan hydrochloride trihydrate IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.4. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{33}H_{38}N_4O_6.HCl, 3H_2O$ in the injection.

Storage. Store protected from light, at a temperature not exceeding 25°.

Iron Dextran Injection

Iron Dextran Injection is a sterile colloidal solution containing a complex of ferric hydroxide with practically hydrolised dextrans of average molecular weight about 1000.

Iron Dextran Injection contains not less than 95.0 per cent and not more than 105.0 per cent w/v of the stated amount of iron and not more than 0.5 per cent of phenol as a preservative.

Category. Haematinic.

Description. A dark brown slightly viscous liquid.

Identification

To 1 ml of Injection on a watch glass, add 2 drops of *ammonium hydroxide*. No precipitate is formed. Add 2 ml of *hydrochloric acid*, and 2 ml of *ammonium hydroxide*. A brown precipitate is formed.

Tests

pH (2.4.24). 4.5 to 7.0.

Chloride content. For products labelled to contain 50 mg per ml of iron: 0.48 per cent to 0.68 per cent; for products labeled to contain 75 or 100 mg per ml of iron: 0.8 per cent to 1.1 per cent.

To 10.0 ml of injection, add 50 ml of *water* and 2 ml of *nitric acid* and titrate immediately with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of Cl.

Phenol. Not more than 0.5 per cent,

Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.2 per cent w/v soluion of *benzyl alcohol* in *methanol.*

Test solution. Mix 5.0 ml of injection and 10.0 ml of the internal standard solution and dilute to 50.0 ml with *water.*

Reference solution (a). A 0.4 per cent w/v soluion of *phenol.*

Reference solution (b). Mix 5.0 ml of reference solution (a) and 10.0 ml of the internal standard solution and dilute to 50.0 ml with *water.*

Chromatographic system

- a fused silica capillary column 30 m x 0.32 mm, packed with macrogol 20000 (film thickness 0.5 µm),
- temperature:
  column. - 150° for 5 minutes, 150° to 230° @ 10° per minute and hold at 230° for 7 minutes,
- inlet port: 200° and detector. 310°,
- flame ionization detector,
- flow rate: 2 ml per minute,using nitrogen as the carrier gas,
- injection volume: 1 µl,
- split ratio: 10:1,
- run time: 20 minutes.

The relative retention time of benzyl alcohol with respect to phenol is about 0.85.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to benzyl alcohol and phenol is not less than 2.0, the tailing factor not more than 2.0 for the phenol peak and the relative standard deviation for replicate injections is not more than 1.0 per cent for the peak response ratio of phenol to benzyl alcohol.

Calculate the content of phenol (C_6H_6O) in the injection.

Non-volatile Residue. For products labeled to contain 50 mg per ml of iron: 28.0 per cent to 32.0 per cent; 75 mg per ml of iron: 35.0 per cent to 40.0 per cent; 100 mg per ml of iron: 37.0 per cent to 43.0 per cent.

Transfer 1.0 ml of injection onto 3 to 5 g of sand spread in a shallow layer in a stainless steel dish, the dish and sand having been previously dried and weighed. Rinse the pipet, with several small portions of *water*, onto the sand. Evaporate on a steam bath to dryness, continue the drying in an oven at 105° for 15 hours, and weigh.

Absorption from Injection Site. Prepare a site over the semitendinosus muscle of one leg of each of two rabbits, each weighing between 1.5 and 2.5 kg, by clipping the fur and disinfecting the exposed skin. Inject each site with a dose of 0.4 ml per kg of body weight in the following manner. Place the needle in the distal end of the semitendinosus muscle at an angle such as to ensure that the full length of the needle is used, then pass it through the sartorius and vastus medialis muscles. House the rabbits separately. Sacrifice the rabbits after 7 days and remove the legs into which the injections were made. Carefully dissect the muscles and examine the site of injection. The muscle is only lightly stained and no heavy black deposit of unabsorbed iron compounds or leakage along fascial planes is observed.

Skin the leg, dissect the flesh from the bone and cut into small pieces. Transfer the pieces to a 1000-ml beaker, add 75 ml of *2 M sodium hydroxide* and sufficient *water* to submerge them, cover the beaker with a watch glass and boil until most of the solid matter has disintegrated. Cool cautiously, add 50 ml of *sulphuric acid,* heat the mixture almost to boiling and add carefully 10 ml of *fuming nitric acid* about 1 ml at a time, until no charring occurs when the excess of nitric acid has been boiled off. Cool, add 175 ml of *water,* boil until solution is complete, cool and dilute to 250.0 ml with *water*. To 5.0 ml of the solution add 3 ml of *sulphuric acid,* heat to fuming and complete the oxidation by adding small quantities of *nitric acid* until the solution is colourless. Cool, add 20 ml of *water,* boil for 3 minutes and add 10 ml of *ammonium citrate solution,* 10 ml of *ammonium thioglycollate solution* followed by *dilute ammonia solution* dropwise until the iron colour is fully developed. Add 1 ml excess of *dilute ammonia solution* and sufficient *water* to produce 100.0 ml. Measure the absorbance of the resulting solution at about 530 nm (2.4.7). For the reference solution, add 10 ml of *ammonium citrate solution,* 10 ml of *ammonium thioglycollate solution* and the same quantities of *dilute ammonia solution* as used above to 20 ml of *water*, and dilute to 100.0 ml. Measure the absorbance of the solution at about 530 nm (2.4.7). From the difference between the absorbances, calculate the amount of Fe present in the legs from a reference curve prepared by treating suitable aliquots of a solution of *ferricammonium sulphate* containing 0.01 per cent w/v of Fe by the above procedure beginning at the words "add 10 ml of *ammonium citrate solution,.....*".

Repeat the determination of Fe on the corresponding legs into which injection was not made beginning at the words "Carefully dissect the muscles....". From the difference between the two amounts of Fe, calculate the proportion of injected iron, as Fe, remaining in the leg tissues. Not more than 20 per cent of the injected iron remains.

Abnormal toxicity. Not less than 500 mg of iron per kg of body weight.

Select five mice, each weighing 18 to 25 g, maintained on an adequately balanced diet. Inject a dose of Injection, equivalent to 200 mg of iron per kg of body weight, into a tail vein at a rate of not more than 0.1 ml per second. Keep the mice under observation for 48 hours after the injection. If none of the mice show outward symptoms of toxicity, the requirements of the test are met. If any of the mice die within the observation

period, select four groups of 10 mice, each weighing 18 to 25 g. Inject, intravenously, all mice of one group with one of the following doses of Injection: 375, 500, 750, or 1000 mg of iron per kg of body weight. Observe the mice for 7 days, and record the number of deaths in each group. If more than 16 mice die, calculate the LD with log-doses and probits. The four doses of Injection, in mg of iron per kg of body weight, are transformed to $x_1 = 2.574$, $x_2 = 2.699$, $x_3 = 2.875$, and $x_4 = 3.000$. The probits corresponding to the number of deaths observed in each group of 10 mice are designated y_1, y_2, y_3 and y_4, respectively, and are given in Table 1 for mortalities from 10 per cent to 90 per cent. For observed deaths of 0 and 10 adjacent to doses giving an intermediate mortality, use the approximate probits 3.02 and 6.98, respectively; omit the end value (at x_1 or x_4) if not adjacent to an intermediate mortality. Since the information in a probit varies with its expectation, assign each probit an approximate relative weight (w) for computing the LD_{50} of the Injection, as shown in Table 2.

Table 2

No. of Deaths	0 or 10	1 or 9	2 or 8	3 or 7	4 to 6
Weight (w)	0.3	0.7	1.0	1.2	1.3

Calculate the weighted means:

$$\bar{x} = \frac{\Sigma(wx)}{\Sigma w}$$

and

$$\bar{y} = \frac{\Sigma(wy)}{\Sigma w}$$

from the sum of the weights, Σw, of the four (or three) acceptable responses and the corresponding weighted sums of the log-doses, $\Sigma(wx)$, and of the probits, $\Sigma(wy)$. From the sums of the weighted products, $\Sigma(wxy)$, and of the weighted squares, $\Sigma(wx^2)$, compute the slope b of the log-dose-probit line as:

$$b = \frac{[\Sigma(wxy) - \bar{x}\Sigma(wy)]}{[\Sigma(wx^2 - \bar{x}\Sigma(wx)]}$$

The LD_{50} for this safety test, in mg of iron per kg of body weight, is calculated as:

$$LD_{50} = \text{antilog}\left[\bar{x} + \frac{(5 - \bar{y})}{b}\right]$$

Bacterial endotoxins (2.2.3). Not more than 0.50 Endotoxin Unit per mg of iron.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay.

For iron — Determine by atomic absorption spectrophotometry (2.4.2), Method B.

Solvent mixture. Dissolve 2.64 g of *calcium chloride dihydrate* in 500 ml of *water*, add 5 ml of *hydrochloric acid* and dilute to 1000.0 ml with *water*.

Test solution. Dilute a volume of injection containing 100 mg of Iron to 200.0 ml with the solvent mixture. Dilute 2.0 ml of the solution to 250.0 ml with the solvent mixture.

Reference solution. A solution of 50 µg per ml of iron prepared by dissolving 350 mg of *ferrous ammonium sulphate hexahydrate* in 1000.0 ml of *water*. Dilute the solution to obtain the concentrations of 1 µg per ml, 2 µg per ml, 3 µg per ml, 4 µg per ml and 5 µg per ml of iron in the solvent mixture.

Set the zero of the instrument using solvent mixture as blank. Measure the absorbance at 248.3 nm using a iron hollow-cathode lamp as source of radiation and an air-acetylene flame.

Table 1

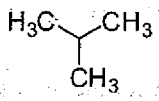
	Probits (normal deviates + 5) Corresponding to Percentages in the Margins									
	0	1	2	3	4	5	6	7	8	9
0	–	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
–	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Calculate the content of Iron, (Fe) in the injection.

Storage. Store at a temperature not exceeding 30°, preserve in single dose and multi dose containers, preferably of Type I or Type II glass.

Labelling. The label states the strength in terms of the equivalent amount of iron, Fe, in a suitable dose-volume per cent.

Isobutane



C_4H_{10} Mol. Wt. 58.1

Isobutane is 2-methylpropane.

Isobutane contains not less than 95.0 per cent of C_4H_{10}.

Category. Pharmaceutical aid.

CAUTION—Isobutane is highly flammable and explosive.

Identification

Determine by infrared absorption spectrophotometry (2.4.6), the solution shows absorption maxima at wavelength of 3.4 µm, 6.8 µm, 7.2 µm, 8.5 µm and 10.9 µm.

Tests

Water (2.3.43). Not more than 0.001 per cent with the following modifications (a) provide the closed-system titrating vessel with an opening through which passes a coarse-porosity gas dispersion tube connected to a sample cylinder; (b) dilute the reagent with *anhydrous methanol* to give a water equivalence factor of between 0.2 and 1.0 mg per ml, age this diluted solution for not less than 16 hours before sanitation; (c) obtain a 100 g sample as directed under inhalation preparation, and introduce the sample into the titration vessel through the gas dispersion tube at a rate of about 100 ml of gas per minute.

High-boiling residues. Not more than 5 µg per ml.

Prepare a cooling coil from copper tubing (about 6 mm outside diameter × about 6.1 m long) to fit into a vacuum-jacketed flask. Immerse the cooling coil in a mixture of dry ice and *acetone* in a vacuum-jacketed flask, and connect one end of the tubing to the propellant sample cylinder. Carefully open the sample cylinder valve, flush the cooling coil with about 50 ml of the propellant, and discard this portion of liquefied propellant. Continue delivering liquefied propellant from the cooling coil, and collect it in a previously chilled 1000-ml sedimentation cone until the cone is filled to the 1000-ml mark. Allow the propellant to evaporate, using a warm water-bath maintained at about 40° to reduce evaporating time. When all of the liquid has evaporated, rinse the sedimentation cone with two 50-ml portions of *pentane*, and combine the rinsings in a tared 150-ml evaporating dish. Transfer 100 ml of the *pentane* solvent to a second tared 150-ml evaporating dish, place both evaporating dishes on a water-bath, evaporate to dryness, and heat the dishes in an oven at 100° for 60 minutes. Cool the dishes in a desiccator, and weigh. Repeat the heating for 15-minute periods until successive weighings are within 0.1 mg, and calculate the weight of the residue obtained from the propellant as the difference between the weights of the residues in the two evaporating dishes.

Acidity of residue. Add 10 ml of *water* to the residue obtained in the test for High boiling residues, mix by swirling for about 30 seconds, add 2 drops of *methyl orange solution*, insert the stopper in the tube, and shake vigorously; no pink or red color appears in the aqueous layer.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Connect one Isobutane cylinder to the chromatograph through a suitable sampling valve and a flow control valve downstream from the sampling valve. Flush the liquid sample through the sampling valve, taking care to avoid entrapment of gas or air in the sampling valve.

Chromatographic system

- an aluminum column 6 m x 3 mm, packed with 10 per cent of liquid phase G30 (Tetraethylene glycol dimethyle ether) on non-acid-washed support S1C (A support prepared from crushed firebrick and calcined or burned with a clay binder above 900° with subsequent acid-wash. It may be silanized),
- temperature:
  column.33°,
- a thermal-conductivity detector,
- flow rate: 50 ml per minute, using nitrogen as the carrier gas.

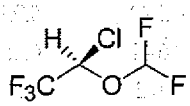
Inject the test solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0.

Inject 2 µl of the reference solution and the test solution.

Calculate the percentage purity by dividing 100 times the Isobutane response by the sum of all of the responses in the chromatogram.

Storage. Store protected from moisture and prevent exposure to excessive heat.

Isoflurane



and enantiomer

$C_3H_2ClF_5O$ Mol. Wt. 184.5

Isoflurane is (2RS)-2-Chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane.

Category. General anaesthetic.

Description. A clear, colourless, mobile and heavy liquid.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoflurane IPRS* or with the reference spectrum of isoflurane.

Tests

Acidity or alkalinity. Shake 20 ml of the sample with 20 ml of *carbon dioxide free water* for 3 minutes and allow to stand. Collect the upper layer and add *0.2 ml of bromocresol* purple solution. Not more than 0.1 ml of *0.01M sodium hydroxide or 0.6 ml of 0.01M hydrochloric acid* is required to change the colour of the indicator.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. The substance under examination.

Reference solution. To 80 ml of *dehydrated alcohol*, add 1.0 ml of the *isoflurane IPRS* and 1.0 ml of *acetone*, avoiding loss by evaporation, dilute to 100.0 ml with *dehydrated alcohol*. Dilute 1.0 ml of the solution to 100.0 ml with the same solvent.

Chromatographic system

- a capillary column 30 m x 0.32 mm, packed with fused silica coated with macrogol 20000 (film thickness 0.25 µm),
- temperature:
  column 35°,
  injector port: 150° and detector port at 250°,
- split ratio: 1:25,
- flame ionization detector,
- flow rate: 1 ml per minute, using helium/ nitrogen as the carrier gas,
- Run time: Untill elution of the *ethanol* peak in the chromatogram obtained with the reference solution.

Inject the 1.0 µl of each solution and 1.0 µl of *dehydrated alcohol* as a blank. The relative retention times with reference to isoflurane (retention time is about 3.8 minutes), for acetone is about 0.75 minute. The test is not valid unless the resolution between the peak due to acetone and the peak due to isoflurane is not less than 5.0 in the chromatogram obtained with reference solution.

Inject the reference solution.The test is not valid unless the relative standard deviation for replicate injections is not more than 15.0 per cent for the peak due to isoflurane.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to the acetone is not more than the area of the principal peak in the chromatogram obtained with reference solution (0.01 per cent); the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (0.01 per cent) ; the sum of the areas of all the secondary peaks is not more than 3.0 times the area of the principal peak in the chromatogram obtained with reference solution (0.03 per cent). Ignore any peak due to isoflurane with an area less than 0.1 times of the area of the principal peak in the chromatogram obtained with reference solution (0.001 per cent).

Chlorides (2.3.12). To 50.0 ml of the sample add 10 ml of *0.01 M sodium hydroxide* and shake for 3 minutes. 5.0 ml of the upper layer complies with the limit test for chloride (10 ppm).

Fluoride. Not more than 10 ppm.

Buffer solution. Dissolve 58.5 g of *sodium chloride*, 57.0 ml of *glacial acetic acid* , 61.5 g of *sodium acetate* and 5.0 g of *cyclohexylene-dinitrilotetra-acetic acid* in *water* and dilute to 500.0 ml with the same solvent. Adjusted the pH 5.0 to 5.5 with a 33.5 per cent w/v solution of *sodium hydroxide* and dilute to 1000.0 ml with *distilled water*.

Determining the end point potentiometrically (2.4.25) for fluoride ion, using a fluoride-selective indicator electrode and a silver-silver chloride reference electrode.

Test solution.Take 10.0 ml of the sample in a separating funnel, add 10 ml of a mixture of 30.0 ml of *dilute ammonia* and 70.0 ml of *water*. Shake for 1 minute and collect the upper layer. Repeat the extraction procedure twice, collecting the upper layer each time. Adjust the combined upper layers to pH 5.2 using *dilute hydrochloric acid* . Add 5.0 ml of *fluoride standard solution (1 ppm)* and dilute to 50.0 ml with *water*. To 20.0 ml of the solution add 20.0 ml of *buffer solution* and dilute to 50.0 ml with *water*.

Reference solutions. To each of 5.0 ml, 4.0 ml, 3.0 ml, 2.0 ml and 1.0 ml of *fluoride standard solution (10 ppm)* add 20.0 ml of a buffer solution and dilute to 50.0 ml with *water*.

Carry out the measurements on 20 ml of each solution.

Calculate the concentration of fluorides using the calibration curve, taking into account the addition of fluoride to the test solution.

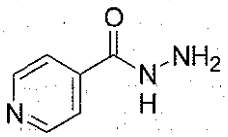
Non-volatile matter. Evaporate 10.0 ml of the sample in a small dish on a water-bath and dry at 50° for 2 hours; the residue weighs not more than 2 mg (0.02 per cent w/v).

Water (2.3.43). Not more than 0.1 per cent w/v, determined on 10.0 ml.

Storage. Store in airtight containers and protected from light, at a temperature below 30°.

Isoniazid

Isonicotinylhydrazid; INH



$C_6H_7N_3O$ Mol. Wt. 137.1

Isoniazid is isonicotinic acid hydrazide.

Isoniazid contains not less than 98.0 per cent and not more than 101.0 per cent of $C_6H_7N_3O$, calculated on the dried basis.

Category. Antituberculosis.

Description. Colourless crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoniazid IPRS* or with the reference spectrum of isoniazid.

B. Dissolve 0.1 g in 2 ml of *water*, add a warm solution of 0.1 g of *vanillin* in 10 ml of *water*, allow to stand and scratch the inside of the container with a glass rod; a yellow precipitate is produced. The precipitate after recrystallisation from 5 ml of *ethanol (70 per cent)* and drying at 105° melts at 226° to 231° (2.4.21).

C. Melts at 170° to 174° (2.4.21).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

pH (2.4.24). 6.0 to 8.0, determined in a 5.0 per cent w/v solution.

Hydrazine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *ethyl acetate*, 20 volumes of *acetone*, 20 volumes of *methanol* and 10 volumes of *water*.

Test solution. Dissolve 1 g of the substance under examination in sufficient of a mixture of equal volumes of *acetone* and *water* to produce 10 ml.

Reference solution. Dissolve 50 mg of *hydrazine sulphate* in 50 ml of *water* and dilute to 100 ml with *acetone*; to 10 ml of the solution add 0.2 ml of the test solution and dilute to 100.0 ml with a mixture of equal volumes of *acetone* and *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Spray with *dimethylaminobenzaldehyde solution* and examine in daylight. The additional spot (due to hydrazine) in the chromatogram obtained with the reference solution is more intense than any corresponding spot in the chromatogram obtained with the test solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100 ml of *water*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 96 volumes of a solution prepared by dissolving 1.4 g *disodium hydrogen phosphate* and 1 ml of *triethylamine* to 1000 ml with *water*, adjusted to pH 6.0 with *orthophosphoric acid* and 4 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject the test solution. Any individual impurity is not more than 0.2 per cent and the sum of all impurities found is not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 25.0 mg of the substance under examination in 50.0 ml of *water*. Dilute 5.0 ml of the solution to 25.0 ml with *water*.

Reference solution. Dissolve 25.0 mg of the *isoniazid IPRS* in 50.0 ml of *water*. Dilute 5.0 ml of the solution to 25.0 ml with *water*.

Use the chromatographic system described under the test for Related substances.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_6H_7N_3O$.

Storage. Store protected from light.

Isoniazid Tablets

Isonicotinylhydrazid Tablets; INH Tablets

Isoniazid Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isoniazid, $C_6H_7N_3O$.

Usual strengths. 50 mg; 100 mg; 300 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Isoniazid with 10 ml of *ethanol (95 per cent)* for 15 minutes, centrifuge and decant the supernatant liquid. Extract the residue with two further quantities, each of 10 ml, of *ethanol (95 per cent)* and evaporate the combined extracts to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoniazid IPRS* or with the reference spectrum of isoniazid.

B. Shake a quantity of the powdered tablets containing 0.1 g of Isoniazid with 50 ml of *ethanol (95 per cent)* and filter. To 5 ml of the filtrate add 0.1 g of *borax* and 5 ml of a 5 per cent w/v solution of *1-chloro-2,4-dinitrobenzene* in *ethanol (95 per cent)*, evaporate to dryness on a water-bath and continue heating for a further 10 minutes. To the residue add 10 ml of *methanol* and mix; a reddish purple colour is produced.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powdered tablets containing 50 mg of Isoniazid, dissolve in 100.0 ml of the mobile phase.

Use the chromatographic system described under Assay.

Inject the test solution. Any individual impurity is not more than 1.0 per cent and the sum of all impurities found is not more than 2.0 per cent.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute suitably with *water* and measure the absorbance of the resulting solution at the maximum at about 263 nm (2.4.7). Calculate the content of $C_6H_7N_3O$ taking 307 as the specific absorbance at 263 nm.

Q. Not less than 80 per cent of the stated amount of $C_6H_7N_3O$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 25.0 mg of Isoniazid and dissolve in 50.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 25.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of the *isoniazid IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Intersil ODS-3),
- mobile phase: mix 96 volumes of a solution prepared by dissolving 1.4 g *disodium hydrogen orthophosphate anhydrous* and 1.0 ml of *triethylamine* to 1000 ml with *water* and adjusted to pH 6.0 with *orthophosphoric acid*, and 4 volumes of *acetonitrile* and filter,
- flow rate: 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

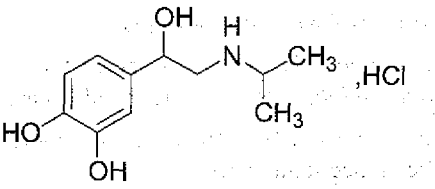
Inject the reference solution and the test solution.

Calculate the content of $C_6H_7N_3O$ in the tablets.

Storage. Store protected from light.

Isoprenaline Hydrochloride

Isoproterenol Hydrochloride



$C_{11}H_{17}NO_3$,HCl Mol. Wt. 247.7

Isoprenaline Hydrochloride is (*RS*)-1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride.

Isoprenaline Hydrochloride contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{11}H_{17}NO_3$,HCl, calculated on the dried basis.

Category. Sympathomimetic.

Description. A white or almost white, crystalline powder. Gradually darkens on exposure to air and light; even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. Aqueous solutions become pink to brownish on standing exposed to air and almost immediately after being made alkaline.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoprenaline hydrochloride IPRS* or with the reference spectrum of isoprenaline hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.5.

C. To 2 ml of a freshly prepared 1 per cent w/v solution add 0.1 ml of *ferric chloride test solution*; an emerald-green colour is produced which, on the gradual addition of *sodium bicarbonate solution*, changes first to blue and then to red.

D. It gives the reactions of chlorides (2.3.1).

Tests

Sulphates (2.3.17). Dissolve 0.5 g in 100 ml of *water*; 15 ml of the resulting solution complies with the limit test for sulphates (0.2 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 5.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A 0.0025 per cent w/v solution of *orciprenaline sulphate IPRS* in the mobile phase.

Reference solution (c). To 5.0 ml of reference solution (a), add 5.0 ml of reference solution (b).

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 5 volumes of *methanol* and 95 volumes of 1.15 per cent w/v solution of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time
Isoprenaline	1.0
Orciprenaline	1.5
Isoprenaline impurity A[1]	1.8

[1]1-(3,4-dihydroxyphenyl)-2-[(1-methylethyl)amino]ethanone.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to isoprenaline and orciprenaline is not less than 3.0.

Inject reference solution (a) and the test solution. Run the chromatogram 7 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak corresponding to isoprenaline impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Weigh 0.5 g, dissolve in 50 ml of *anhydrous glacial acetic acid* with the aid of the minimum heat, cool and add 15 ml of *mercuric acetate solution* and titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02477 g of $C_{11}H_{17}NO_3,HCl$.

Storage. Store protected from light.

Isoprenaline Injection

Isoprenaline Hydrochloride Injection; Isoproterenol Injection; Isoproterenol Hydrochloride Injection

Isoprenaline Injection is a sterile solution of Isoprenaline Hydrochloride in Water for Injections. It may contain suitable stabilising agents.

Isoprenaline Hydrochloride Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isoprenaline hydrochloride, $C_{11}H_{17}NO_3,HCl$.

Usual strength. 200 µg per ml.

Description. A colourless or very pale yellow solution.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *2-propanol*, 16 volumes of *water* and 4 volumes of *strong ammonia solution*.

Test solution. Use the injection, diluted if necessary with sufficient *methanol (80 per cent)* to produce a solution containing 0.02 per cent w/v of isoprenaline hydrochloride.

Reference solution. A solution containing 0.02 per cent w/v of *isoprenaline hydrochloride IPRS* in *methanol (80 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, place it for a few minutes in an atmosphere saturated with *diethylamine* and spray with *diazotised nitroaniline solution*. The chromatogram obtained with the test solution exhibits an elongated zone corresponding to that obtained with the reference solution.

B. To 2 ml add 0.1 ml of *ferric chloride test solution*; an emerald-green colour develops which, on gradual addition of *sodium bicarbonate solution*, changes to blue and then to red.

Tests

pH (2.4.24). 2.5 to 3.0.

Bacterial endotoxins (2.2.3). Not more than 1250.0 Endotoxin Units per mg of isoprenaline hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing 2 mg of Isoprenaline Hydrochloride to 100.0 ml with 0.1 per cent w/v of *sodium metabisulphite*.

Reference solution (a). A 0.002 per cent w/v solution of *isoprenaline hydrochloride IPRS* in a 0.1 per cent w/v of *sodium metabisulphite*.

Reference solution (b). A mixture of 1 volume of a 0.02 per cent w/v solution of *adrenaline acid tartrate* in the mobile phase containing 1.0 per cent w/v of *sodium metabisulphite* and 18 volumes of reference solution (a).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 to 10 µm) (Such as Nucleosil C18),
- mobile phase: dissolve 1.76 g of *sodium heptanesulphonate* in 800 ml of *water*, add 200 ml of *methanol* and adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.5.

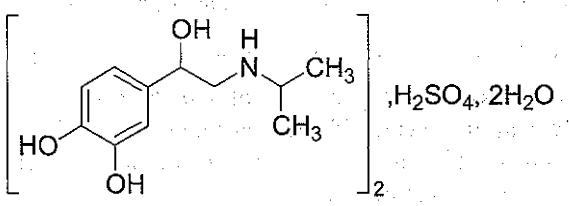
Inject reference solution (a) and the test solution.

Calculate the content of $C_{11}H_{17}NO_3$,HCl in the injection.

Storage. Store protected from light at a temperature not exceeding 30°.

Isoprenaline Sulphate

Isoproterenol Sulphate



$(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$ Mol. Wt. 556.6

Isoprenaline Sulphate is (*RS*)-1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol sulphate dihydrate.

Isoprenaline Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{11}H_{17}NO_3)_2,H_2SO_4$, calculated on the anhydrous basis.

Category. Sympathomimetic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Dissolve 0.5 g in 1.5 ml of *water*, add 3.5 ml of *2-propanol* scratch the walls of the container with a glass rod to induce crystallisation, collect the crystals and dry over *phosphorus pentoxide* at 60° at a pressure of 1.5 to 2.5 kPa. The crystals comply with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoprenaline sulphate IPRS* treated in the same manner or or with the reference spectrum of isoprenaline.

B. To 1 ml of a 1 per cent w/v solution add 0.1 ml of *ferric chloride test solution*; an emerald-green colour is produced which, on gradual addition of *sodium bicarbonate solution*, changes first to blue and then to red.

C. To 5 ml of a freshly prepared 1 per cent w/v solution add 0.15 ml of *silver nitrate solution*; a greyish precipitate is produced on standing for 10 minutes and the solution becomes pink.

D. A 10 per cent w/v solution gives the reaction of sulphates (2.3.1).

Tests

Appearance of solution. A freshly prepared 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 4.0 to 5.5, determined in a freshly prepared 1.0 per cent w/v solution in *carbon dioxide-free water*.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 5.0 to 7.5 per cent determined on 0.2 g.

Assay. Dissolve 0.8 g in 40 ml of *anhydrous glacial acetic acid*, warming gently if necessary and titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05206 g of $(C_{11}H_{17}NO_3)_2,H_2SO_4$.

Storage. Store protected from light.

Isoprenaline Tablets

Isoprenaline Sulphate Tablets; Isoproterenol Tablets; Isoproterenol Sulphate Tablets

Isoprenaline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isoprenaline sulphate, $(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$.

Usual strength. 10 mg.

Identification

A. Extract a quantity of the powdered tablets containing about 50 mg of Isoprenaline Sulphate with 5 ml of *water* and filter. Reserve the filtrate for test C. To 1 ml of the filtrate add 0.1 ml of *ferric chloride test solution*; an emerald-green colour is produced which, on gradual addition of *sodium bicarbonate solution*, changes first to blue and then to red.

B. Extract a quantity of the powdered tablets containing about 50 mg of Isoprenaline Sulphate with 5 ml of *water* and filter. To the filtrate add 0.15 ml of *silver nitrate solution*; a greyish precipitate is produced on standing for 10 minutes and the solution becomes pink.

C. To 2 ml of the filtrate reserved from test A add 0.5 ml of *dilute hydrochloric acid* and 0.5 ml of *barium chloride solution*; a white precipitate is formed.

Tests

Uniformity of content. Complies with the test stated under Tablets.

Crush one tablet and shake with 50 ml of *water* for 15 minutes. Add sufficient *water* to produce 100.0 ml, mix and filter. To 20.0 ml of the filtrate add 0.5 ml of *ferrous sulphate-citrate solution* and 2 ml of *glycine buffer solution* and allow to stand for 20 minutes. Dilute to 25.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7). Calculate the content of $(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$ in the tablet from the absorbance obtained by repeating the determination using 2.0 ml of a 0.1 per cent w/v solution of *isoprenaline sulphate IPRS* in place of the substance under examination.

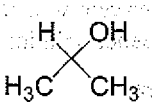
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.1 g of Isoprenaline Sulphate and shake with 50 ml of *water* for 15 minutes. Add sufficient *water* to produce 100.0 ml, mix and filter. Dilute 20.0 ml of the filtrate to 200.0 ml with *water*. To 20.0 ml of the resulting solution add 0.5 ml of *ferrous sulphate-citrate solution* and 2 ml of *glycine buffer solution* and allow to stand for 20 minutes. Dilute to 25.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7). Calculate the content of $(C_{11}H_{17}NO_3)_2,H_2SO_4,2H_2O$ from the absorbance obtained by repeating the determination using 2.0 ml of a 0.1 per cent w/v solution of *isoprenaline sulphate IPRS* in place of the substance under examination.

Storage. Store protected from light.

Isopropyl Alcohol

Propan-2-ol



C_3H_8O Mol. Wt. 60.1

Isopropyl Alcohol is propan-2-ol.

Isopropyl Alcohol contains not less than 99.0 per cent v/v of C_3H_8O.

Category. Pharmaceutical aid (solvent).

Description. A clear and colourless liquid.

Identification

Tests A and D may be omitted if tests B and C are carried out. Test C may be omitted if tests A, B and D are carried out.

A. Relative density (See test).

B. Refractive index (See test).

C. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isopropyl alcohol IPRS* or with the reference spectrum of isopropyl alcohol.

D. To 1ml, add 4 ml of *water* and mix. Carefully add 2 ml of 1 per cent w/v solution of *dimethylaminobenzaldehyde* in *sulphuric acid*, ensuring that the liquids do not mix; a bright reddish-violet ring forms immediately at the junction of the 2 liquids. After 2 to 5 minutes, the entire *sulphuric acid* layer turns violet.

Tests

Appearance of solution. Isopropyl Alcohol is clear (2.4.1) and colourless (2.4.1). A 5 per cent v/v solution of Isopropyl Alcohol in *water* remains clear (2.4.1) after 5 minute.

Acidity or alkalinity. Gently boil 25 ml of Isopropyl Alcohol for 5 minutes with 25 ml of *carbon dioxide-free water* and cool, protected from carbon-dioxide in the air. Add 0.1 ml of *phenolphthalein solution*. The solution is colourless. Not more than 0.6 ml of *0.01M sodium hydroxide* is required to change the colour of the solution to pale pink.

Absorbance (2.4.7). When examined at the wavelength 230 nm, 250 nm, 270 nm, 290 nm and 310 nm, Isopropyl Alcohol shows an absorption maximum 0.30, 0.10, 0.03, 0.02 and 0.01 respectively.

The absorbance is measured between 230 nm and 310 nm using *water* as the compensation liquid. The spectrum shows a steadily descending curve with no observable peaks or shoulders.

Refractive index (2.4.27). 1.376 to 1.379, determined at 20°.

Relative density (2.4.29). 0.785 to 0.789, determined at 20°.

Peroxides. Place 8 ml of *potassium iodide* and *starch solution* in a 12-ml glass-stoppered cylinder of about 1.5 cm diameter. Fill completely with Isopropyl Alcohol, insert the stopper, shake vigorously and allow to stand in the dark for 30 minutes, no colouration is produced.

Benzene and related substances. Determine by gas chromatography (2.4.13).

Test solution (a). Isopropyl Alcohol.

Test solution (b). A 0.1 per cent v/v solution of *2-butanol* in test solution (a).

Reference solution (a). A solution containing 0.1 per cent, v/v each, of *2-butanol* and *1 -propanol* in test solution (a).

Reference solution (b). Dilute 0.1 ml of *benzene* to 100.0 ml with test solution (a). Dilute 0.2 ml of the solution to 100.0 ml with test solution (a).

Chromatographic system

- a fused silica column 30 m × 0.32 mm, packed with 6.0 per cent cyanopropylphenyl and 94 per cent dimethylpolysiloxane (film thickness 1.8 µm) (Such as DB-624),
- temperature:
  column. 40° hold for 12 minutes, 40° to 240° @ 10° per minute and hold at 240° for 10 minutes,
  inlet port 280° and detector at 280°,
- split ratio: 1:5,
- flame ionization detector,
- linear velocity: 35 cm per second using nitrogen as the carrier gas,
- injection volume: 1 µl.

The retention time of benzene is about 10 minutes.

Inject reference solution (a). The test is not valid unless the resolution between the peak due to *1-propanol* and *2-butanol* is not less than 10.

Inject reference solution (b) and test solution (a). In the chromatogram obtained with test solution (a) the area of any peak corresponding to benzene is not more than 0.5 times the area of the benzene peak obtained with reference solution (b) (2 ppm). Record the chromatogram adjusting the sensitivity so that the height of the peak due to benzene represents at least 10 per cent of full-scale deflection.

Inject reference solution (a) and test solution (b). In the chromatogram obtained with test solution (b), the area of all secondary peaks other than 2-butanol is not more than 3 times the area of the peak due to 2- butanol in the chromatogram obtained with reference solution (a) (0.3 per cent). Record the chromatogram adjusting the sensitivity so that the height of the 2 peaks following the principal peak in the chromatogram obtained with reference solution (a) represents at least 50 per cent of full-scale deflection.

Non-volatile substances. Not more than 20 ppm.

Evaporate 100 g on a water-bath after having verified that it complies with the test for peroxides and dry the residue at 105°. The residue weigh is not more than 2.0 mg.

Water (2.3.43). Not more than 0.5 per cent, determined on 5.0 g.

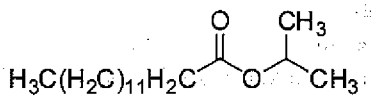
Assay. Determine by gas chromatography (2.4.13), as described under Benzene and related substances.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to 1-propanol and 2-butanol is not less than 10.

Inject test solution (a). Calculate the content of C_3H_8O by area normalization.

Storage. Store protected from light.

Isopropyl Myristate



$C_{17}H_{34}O_2$ Mol. Wt. 270.5

Isopropyl Myristate is isopropyl tetradecanoate.

Isopropyl Myristate contains not less than 90.0 per cent of $C_{17}H_{34}O_2$.

Category. Pharmaceutical aid.

Description. A clear, colourless, oily liquid.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Superpose 2 ml of a 0.1 per cent w/v solution in *ethanol (95 per cent)* on a freshly prepared solution of 20 mg of *dimethylaminobenzaldehyde* in 2.0 ml of *sulphuric acid*. After 2 minutes, a yellowish-red colour appears at the junction of the 2 liquids and gradually becomes red.

C. Complies with the test for saponification value (2.3.37).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

Refractive index (2.4.27). 1.434 to 1.437.

Relative density (2.4.29). About 0.853.

Acid value (2.3.23). Not more than 1.0.

Saponification value (2.3.37). 202 to 212.

Iodine value (2.3.28). Not more than 1.0.

Total ash (2.3.19). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.1 per cent, determined on 5.0 g.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. Dissolve about 50 mg of *tricosane* in 250.0 ml of *heptane*.

Test solution. Dissolve about 20 mg of the substance under examination in 100.0 ml of the internal standard solution.

Reference solution. A 0.02 per cent w/v solution of *isopropyl tetradecanoate IPRS* in internal standard solution.

Chromatographic system

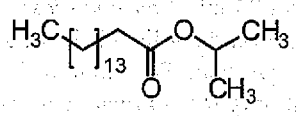
- a fused silica column 50 m x 0.2 mm, packed with poly(cyanopropyl) siloxane (film thickness 0.2 µm),
- temperature:
  column. 185°,
  inlet port and detector at 250°,
- flame ionization detector,
- flow rate: 1 ml per minute, using nitrogen as the carrier gas.

Inject 2 µl of the reference solution and the test solution.

Calculate the content of $C_{17}H_{34}O_2$.

Storage. Store protected from light.

Isopropyl Palmitate



$C_{19}H_{38}O_2$ Mol. Wt. 298.5

Isopropyl Palmitate is Isopropyl hexadecanoate.

Isopropyl Palmitate contains not less than 90.0 per cent of $C_{19}H_{38}O_2$.

Category. Excipient.

Description. A clear, colourless, oily liquid.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Superpose 2 ml of a 0.1 per cent w/v solution in *ethanol (95 per cent)* on a freshly prepared solution of 20 mg of *dimethylaminobenzaldehyde* in 2 ml of *sulphuric acid*. After 2 minutes, a yellowish-red colour appears at the junction of the 2 liquids which gradually becomes red.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

Refractive index (2.4.27). 1.436 to 1.440.

Viscosity (2.4.28). 5 mPas to 10 mPas.

Relative density (2.4.29). About 0.854.

Total ash (2.3.19). Not more than 0.1 per cent.

Acid value (2.3.23). Not more than 1.0.

Iodine value (2.3.28). Not more than 1.0.

Saponification value (2.3.37). 183 to 193.

Water (2.3.43). Not more than 0.1 per cent, determined on 5.0 g.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. Dissolve 50 mg of *tricosane* in *heptane* and dilute to 250.0 ml with *heptane*.

Test solution. Dissolve 20 mg of the substance under examination in the internal standard solution and dilute to 100.0 ml with the same solution.

Reference solution. A 0.02 per cent w/v solution of *isopropyl hexadecanoate IPRS* in the internal standard solution.

Chromatographic system
- a capillary column 50 m x 0. 2 mm, packed with *poly(cyanopropyl)siloxane* (film thickness 0.2 µm),
- temperature:

column	time (min)	temperature (°)
	0-6	125→185
	6-16	185

- Inlet port and detector at 250°,
- flame ionization detector,
- split ratio: 1:40,
- flow rate: 1 ml per minute, using nitrogen as the carrier gas.

Inject 2 µl of the reference solution and the test solution.

Calculate the content of $C_{19}H_{38}O_2$.

Storage. Store protected from light.

Isopropyl Rubbing Alcohol

Isopropyl Rubbing Alcohol contains not less than 68.0 per cent v/v and not more than 72.0 per cent v/v of isopropyl alcohol, C_3H_8O with the remainder consisting of *water*, with or without suitable stabilizers, perfume oil and colour permitted for the use in drugs.

Category. Skin disinfectant.

Tests

Specific gravity (2.4.29). 0.872 to 0.883 at 20°.

Acidity. Transfer 50 ml to a suitable flask and add about 75 ml of *carbon dioxide-free water* and titrate with *0.02 M sodium hydroxide* potentiometrically (2.4.25) to a pH of 8.5; not more than 1.0 ml of *0.02 M sodium hydroxide* is used.

Limit of nonvolatile residue. Not more than 5 mg (0.01 per cent). Evaporate 50 ml to dryness in a tared porcelain dish on a steam-bath and dry at 105° for 1 hour.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.7 per cent v/v solution of *1-propanol* in *water*.

Test solution. Dilute 1.0 ml of the solution under examination to 100.0 ml with the internal standard solution.

Reference solution. A 0.7 per cent v/v solution of *isopropyl alcohol* in the internal standard solution.

Chromatographic system
- a capillary column 30 m × 0.25 mm, packed with 6.0 per cent cyanopropylphenyl and 94 per cent dimethylpolysiloxane (film thickness 1.4 µm) (Such as DB-624),
- temperature:
  column 50° for 2 minutes, 50° to 240° @ 20° per minute and hold at 240° for 2 minutes,
- inlet port 180° and detector at 280°,
- flame ionization detector,
- split ratio: 25:1;
- flow rate 0.5 ml per minute, using nitrogen as the carrier gas,
- injection volume: 1 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to isopropyl alcohol and the internal standard is not less than 2.0 and the relative standard deviation of peak area ratio due to isopropyl alcohol and the internal standard for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

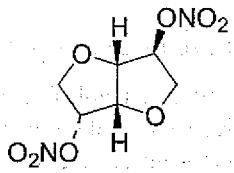
Calculate the content of C_3H_8O using ratio of the peak area of isopropyl alcohol to that of peak area of the internal standard.

Storage. Store protected from heat and preserve in tight containers.

Labelling. Label it to indicate that it is flammable.

Diluted Isosorbide Dinitrate

Diluted Sorbide Dinitrate; Diluted Sorbide Nitrate



$C_6H_8N_2O_8$ Mol. Wt. 236.1

Diluted Isosorbide Dinitrate is a dry mixture of 1,4:3, 6-dianhydro-D-glucitol 2,5-dinitrate with Lactose, Mannitol or other suitable inert diluent. It may contain a suitable stabilising agent.

Diluted Isosorbide Dinitrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isosorbide dinitrate, $C_6H_8N_2O_8$ and usually contains 20 per cent to 50 per cent of isosorbide dinitrate.

Category. Antianginal.

Description. A fine, white, crystalline powder.

CAUTION — Undiluted isosorbide dinitrate is a powerful explosive and can be exploded with percussion or excessive heat. Proper precautions must be taken in handling it and only exceedingly small amounts should be isolated.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution (b).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. Toluene.

Test solution. Extract a quantity containing 2 mg of isosorbide dinitrate with 1 ml of *ether* and centrifuge.

Reference solution. Prepare in the same manner as the test solution but using *diluted isosorbide dinitrate IPRS* in place of the substance under examination.

Apply to the plate 20 µl of each solution. After development, dry the plate in a current of air, spray with a 1 per cent w/v solution of *diphenylamine* in *methanol* and expose for 15 minutes to ultraviolet light at 254 and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Extract a quantity containing 10 mg of isosorbide dinitrate with 10 ml of *ether* and filter. Evaporate the filtrate to dryness at a temperature not exceeding 35° and dissolve the residue in 0.15 ml of *sulphuric acid (50 per cent)* containing a trace of *diphenylamine*; an intense blue colour is produced.

Tests

Inorganic nitrates. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

Mobile phase. A mixture of 60 volumes of *toluene*, 30 volumes of *acetone* and 15 volumes of *glacial acetic acid.*

Test solution. A solution of the substance under examination in *ethanol (95 per cent)* containing the equivalent of 2.0 per cent w/v of isosorbide dinitrate.

Reference solution. Prepare freshly a 0.01 per cent w/v solution of *potassium nitrate* in *ethanol (90 per cent).*

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide* and *starch solution.* Expose the plate to ultraviolet light at 254 nm for 15 minutes. Examine in daylight. Any spot corresponding to potassium nitrate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay, changing the detector setting to 210 to 215 nm and using injection volume of 10 µl.

Under the stated conditions, the retention times are: isosorbide dinitrate, about 5 minutes; isosorbide 2-nitrate, about 8 minutes; isosorbide 5-nitrate, about 11 minutes.

Inject reference solution (c). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (c) is not less than 20 per cent of the full scale of the recorder.

Inject reference solution (e). The test is not valid unless in the chromatogram obtained with reference solution (e), the resolution between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

Inject test solution (a), reference solution (c) and reference solution (d). In the chromatogram obtained with test solution (a) the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent); the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Mix a weighed quantity of the substance under examination containing about 25 mg of isosorbide dinitrate with 20 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the mobile phase. Filter the solution through a suitable membrane filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution (a). Mix a quantity of *diluted isosorbide dinitrate IPRS* containing 25.0 mg of isosorbide dinitrate with 20 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the mobile phase. Filter the solution through a suitable membrane filter.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c). Dissolve 10.0 mg of *isosorbide 2-nitrate IPRS* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 0.1 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (d). Dissolve 10.0 mg of *isosorbide mononitrate IPRS (isosorbide-5-nitrate RS)* in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 0.1 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (e). Dissolve 5 mg of *isosorbide 2-nitrate IPRS* in the mobile phase and dilute to 10 ml with the mobile phase. To 1 ml of the solution add 0.5 ml of reference solution (a) and dilute to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with aminopropylmethylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 15 volumes of *ethanol* and 85 volumes of *trimethylpentane*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained is not less than 50 per cent of the full scale of the recorder. If the areas of the peaks from two successive injections do not agree to within 1.0 per cent, then inject a further four times and calculate, for the six injections, the relative standard deviation. The assay is not valid unless the relative standard deviation for the six injections is at most 2.0 per cent.

Inject alternately test solution (b) and reference solution (b).

Calculate the content of isosorbide dinitrate as a percentage of the declared content.

Storage. Store protected from light, at a temperature not exceeding 15°.

Labelling. The label states the percentage content of isosorbide dinitrate.

Isosorbide Dinitrate Tablets

Sorbide Dinitrate Tablets; Sorbide Nitrate Tablets

Isosorbide Dinitrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isosorbide dinitrate, $C_6H_8N_2O_8$.

Usual strengths. The equivalent of 5 mg and 10 mg of isosorbide dinitrate.

Identification

CAUTION — Undiluted isosorbide dinitrate is a powerful explosive and can be exploded with percussion or excessive heat. Proper precautions must be taken in handling it and only exceedingly small amounts should be isolated.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. Toluene.

Test solution. Extract a quantity of the powdered tablets containing 2 mg of isosorbide dinitrate with 1 ml of *ether* and centrifuge.

Reference solution. Dissolve 2 mg of *diluted isosorbide dinitrate IPRS* in 1 ml of *ether*.

Apply to the plate 20 µl of each solution. After development, dry the plate in a current of air, spray with a 1 per cent w/v solution of *diphenylamine* in *methanol* and expose for 15 minutes to ultraviolet light at 254 nm and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution

B. Extract a quantity of the powdered tablets containing 10 mg of isosorbide dinitrate with 10 ml of *ether* and filter. Evaporate the filtrate to dryness at a temperature not exceeding 35° and dissolve the residue in 0.15 ml of *sulphuric acid (50 per cent)* containing a trace of *diphenyl-amine*; an intense blue colour is produced.

Tests

Dissolution (2.5.2). (for tablets intended to be swallowed whole).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw 10 ml of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution of *diluted isosorbide dinitrate IPRS* in the dissolution medium containing the same concentration of isosorbide dinitrate as that expected in the dissolution medium in the vessel.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of equal volumes of *methanol* and *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 222 nm.
- injection volume: 100 µl.

Calculate the content of $C_6H_8N_2O_8$.

Q. Not less than 80 per cent of the stated amount of $C_6H_8N_2O_8$.

Inorganic nitrates. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 60 volumes of *toluene*, 30 volumes of *acetone* and 15 volumes of *glacial acetic acid*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of isosorbide nitrate with 5 ml of *ethanol (95 per cent)* and filter.

Reference solution. Prepare freshly a 0.01 per cent w/v solution of *potassium nitrate* in *ethanol (90 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide* and *starch solution*. Expose the plate to ultraviolet light at 254 nm for 15 minutes. Examine in daylight. Any spot corresponding to potassium nitrate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Add 20 ml of the mobile phase to a quantity of the powdered tablets containing 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the same solvent. Filter through a glass-fibre filter (such as Whatman GF/C) and use the filtrate.

Reference solution (a). A solution containing 0.0005 per cent w/v of *isosorbide 2-nitrate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v of *isosorbide mononitrate IPRS* in the mobile phase.

Reference solution (c). A solution containing 0.005 per cent w/v, each of, *diluted isosorbide dinitrate IPRS* and *isosorbide 2-nitrate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with aminopropylmethylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 15 volumes of *ethanol* and 85 volumes of *2,2,4-trimethylpentane*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless, in the chromatogram obtained, the resolution between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

In the chromatogram obtained with the test solution the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the area of any peak corresponding to isosorbide 5-nitrate is not greater than the area of the principal peak obtained with reference solution (b) (0.5 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using following modifications.

Test solution. Disperse 1 tablet in 20 ml of the mobile phase and dilute to obtain a concentration equivalent to 0.01 per cent w/v of isosorbide dinitrate in the same solvent.

Reference solution. A solution *diluted isosorbide dinitrate IPRS* equivalent to 0.01 per cent w/v of isosorbide dinitrate in the mobile phase.

Inject the reference solution and the test solution.

Calculate the content of $C_6H_8N_2O_8$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Add 20 ml of the mobile phase to a weighed quantity of the powdered tablets containing about 25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the same solvent. Filter through a glass-fibre filter (such as Whatman GF/C) and dilute 1 volume to 10 volumes with the mobile phase.

Reference solution (a). Add 20 ml of the mobile phase to a quantity of *diluted isosorbide dinitrate IPRS* containing

25 mg of isosorbide dinitrate, mix with the aid of ultrasound for 15 minutes and dilute to 25 ml with the mobile phase. Filter through a glass-fibre filter (such as Whatman GF/C) and dilute 1.0 ml to 10.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v, each of, *diluted isosorbide dinitrate IPRS* and *isosorbide 2-nitrate IPRS* in the mobile phase.

Use chromatographic system as described under Related substances, using a detection wavelength of 230 nm.

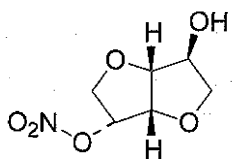
The test is not valid unless, in the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to isosorbide dinitrate and isosorbide 2-nitrate is at least 6.0.

Calculate the content of $C_6H_8N_2O_8$ in the tablets.

Storage Store at a temperature not exceeding 30°.

Labelling. The label states whether the tablets are to be swallowed whole, chewed before swallowing or allowed to dissolve in the mouth.

Diluted Isosorbide Mononitrate



$C_6H_9NO_6$ Mol. Wt. 191.1

Diluted Isosorbide Mononitrate is a dry mixture of isosorbide mononitrate and lactose monohydrate or mannitol.

Diluted Isosorbide Mononitrate is 1,4:3,6-dianhydro-D-glucitol 5-nitrate.

Diluted Isosorbide Mononitrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of $C_6H_9NO_6$.

Category. Antianginal.

Description. Undiluted isosorbide mononitrate is a white, crystalline powder.

Identification

A. Shake a quantity containing 25 mg of isosorbide mononitrate with 15 ml of *acetone* for 2 minutes. Filter, evaporate the filtrate to dryness at a temperature not exceeding 35° and dry the residue over *phosphorus pentoxide* at a pressure of 0.7 kPa for 16 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isosorbide mononitrate IPRS* treated in the same manner or with the reference spectrum of isosorbide mononitrate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *methanol* and 95 volumes of *dichloromethane*.

Test solution. Shake a quantity of the substance under examination containing 10 mg of isosorbide mononitrate with 10 ml of *ethanol (95 per cent)* and filter.

Reference solution. A 0.1 per cent w/v solution of *isosorbide mononitrate IPRS* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air, spray with a freshly prepared *potassium iodide* and *starch solution* and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 10 volumes of *water*, 15 volumes of *methanol*, 25 volumes of *anhydrous acetic acid* and 50 volumes of *dichloroethane*.

Test solution. Shake a quantity of the substance under examination containing 0.1 g of *lactose* or *mannitol* with 10 ml of *water*.

Reference solution (a). Dissolve 0.1 g of *lactose* in 10 ml of *water*.

Reference solution (b). Dissolve 0.1 g of *mannitol* in 10 ml of *water*.

Reference solution (c). Mix equal volumes of reference solution (a) and (b).

Apply to the plate 1 µl of each solution. After development, dry the plate in a current of warm air. Repeat immediately the development after renewing the mobile phase. Dry the plate in a current of warm air. Spray with *4-aminobenzoic acid solution*. Dry the plate in a cold air until the *acetone* is removed. Heat the plate at 100° for 15 minutes. Allow to cool and spray with a 0.2 per cent w/v solution of *sodium periodate*. Dry the plate in a current of cold air. Heat the plate at 100° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a) for lactose or to the principal spot in the chromatogram obtained with reference solution (b) for mannitol. The identification is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Tests

Inorganic nitrates. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene*.

Test solution. Shake a quantity of the substance under examination containing 0.1 g of isosorbide mononitrate with 5 ml of *ethanol (95 per cent)* and filter.

Reference solution. Dissolve 10 mg of *potassium nitrate* in 1 ml of *water* and dilute to 100 ml with *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray with a freshly prepared *potassium iodide* and *starch solution* and examine under ultraviolet light at 254 nm. Any spot corresponding to nitrate ion in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent, calculated as potassium nitrate).

Isosorbide dinitrate and isosorbide 2-nitrate. Determine by liquid chromatography (2.4.14) as described under Assay, changing the detection to 210 nm to 215 nm and using injection volume of 10 µl.

Under the stated conditions, the retention times are: isosorbide dinitrate about 5 minutes, isosorbide-2-nitrate about 8 minutes and isosorbide 5-nitrate about 11 minutes.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is at least 20 per cent of the full scale of the recorder.

Inject reference solution (d). The test is not valid unless in the chromatogram obtained, the resolution between the peaks corresponding to isosorbide 2-nitrate and isosorbide 5-nitrate is at least 4.0.

Inject test solution (a), reference solution (b) and reference solution (c). In the chromatogram obtained with test solution (a), the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak corresponding to isosorbide dinitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Weigh a quantity of the substance under examination containing 25 mg of isosorbide mononitrate, dissolve in 25.0 ml of the mobile phase and filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *isosorbide mononitrate IPRS* in the mobile phase.

Reference solution (b). A 0.0005 per cent w/v solution of *isosorbide-2-nitrate IPRS* in the mobile phase.

Reference solution (c). A 0.0005 per cent w/v solution of *isosorbide dinitrate IPRS* in the mobile phase.

Reference solution (d). A solution containing 0.005 per cent w/v, each of, *isosorbide mononitrate IPRS (isosorbide-5-nitrate IPRS)* and *isosorbide-2-nitrate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with aminopropylmethylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 15 volumes of *ethanol* and 85 volumes of *trimethylpentane*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and test solution (b).

Calculate the content of $C_6H_9NO_6$.

Storage. Store protected from light.

Labelling. The label states the percentage content of isosorbide mononitrate.

Isosorbide Mononitrate Tablets

Sorbide Mononitrate Tablets

Isosorbide Mononitrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of $C_6H_9NO_6$.

Usual strengths. 10 mg; 20 mg; 40 mg; 60 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *methanol* and 95 volumes of *dichloromethane*.

Test solution. Extract a quantity of the powdered tablets containing 10 mg of isosorbide mononitrate with 10 ml of *ethanol (95 per cent)* and centrifuge.

Reference solution. Dissolve 10 mg of *isosorbide mononitrate IPRS* in 10 ml of *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air, spray with a 1 per cent w/v solution of *diphenylamine* in *methanol* and expose for 15 minutes to ultraviolet at 254 nm and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw 10 ml of the medium and filter promptly, rejecting the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution of *isosorbide mononitrate IPRS* in the dissolution medium containing the same concentration of isosorbide mononitrate as that expected in the dissolution medium in the vessel.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with endcapped octyldecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 2),
- mobile phase: a mixture of equal volumes of *methanol* and *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 100 µl.

Calculate the content of $C_6H_9NO_6$.

Q. Not less than 80 per cent of the stated amount of $C_6H_9NO_6$.

Inorganic Nitrates. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 60 volumes of *toluene*, 30 volumes of *acetone* and 15 volumes of *glacial acetic acid*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of isosorbide mononitrate with 5 ml of *ethanol (95 per cent)* and filter.

Reference solution. Prepare freshly a 0.01 per cent w/v solution of *potassium nitrate* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of air until the acetic acid is completely removed. Spray copiously with freshly prepared *potassium iodide and starch solution*. Expose the plate to ultraviolet light at 254 nm for 15 minutes and examine in daylight. Any spot corresponding to *potassium nitrate* in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 50 mg of isosorbide mononitrate with 15 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 25.0 ml with the same solvent and filter through a membrane filter with a nominal pore size not exceeding 0.45 µm.

Reference solution (a). A solution containing 0.001 per cent w/v of *isosorbide 2-nitrate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.001 per cent w/v of *isosorbide dinitrate IPRS* in the mobile phase.

Reference solution (c). A solution containing 0.001 per cent, each of, *isosorbide 2-nitrate IPRS* and *isosorbide mononitrate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with endcapped octyldecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 2),
- mobile phase: 30 volumes of *methanol* and 70 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless, in the chromatogram obtained, the resolution between isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

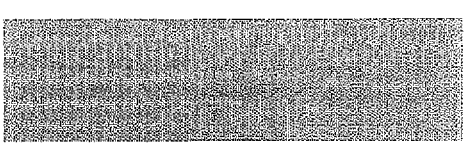
In the chromatogram obtained with the test solution the area of any peak corresponding to isosorbide 2-nitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the area of any peak corresponding to isosorbide dinitrate is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using following modification.

Test solution. Disperse 1 tablet in 20 ml of the mobile phase and dilute to obtain a concentration of 0.005 per cent w/v of isosorbide mononitrate in the same solvent.

Reference solution. A 0.005 per cent w/v solution of *isosorbide mononitrate IPRS* in the mobile phase.



Inject the reference solution and the test solution.

Calculate the content of $C_6H_9NO_6$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of isosorbide mononitrate, add 70 ml of the mobile phase, mix with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the mobile phase. Centrifuge. Mix 1 volume of the supernatant liquid with 5 volumes of the mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm.

Reference solution (a). Add 20 ml of the mobile phase to a weighed quantity of *isosorbide mononitrate IPRS* containing 25 mg of isosorbide mononitrate, mix with the aid of ultrasound for 15 minutes, dilute to 25.0 ml with the mobile phase, mix and centrifuge. Dilute 1.0 ml of the clear supernatant liquid to 10.0 ml with the mobile phase and filter through a membrane filter with a nominal pore size not greater than 0.45 µm.

Reference solution (b). A solution containing 0.001 per cent w/v, each of, *isosorbide mononitrate IPRS* and *isosorbide 2-nitrate IPRS* in the mobile phase.

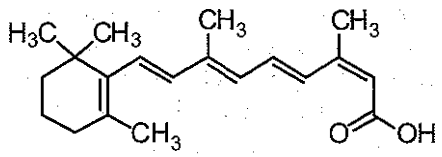
Use the chromatographic system described under test for Related substances.

The test is not valid unless in the chromatogram obtained with reference solution (b), the resolution between the peaks corresponding to isosorbide mononitrate and isosorbide 2-nitrate is at least 2.4.

Calculate the content of $C_6H_9NO_6$ in the tablets.

Storage. Store at a temperature not exceeding 30°.

Isotretinoin



$C_{20}H_{28}O_2$ Mol. Wt. 300.4

Isotretinoin is (2*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid.

Isotretinoin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{28}O_2$, calculated on the dried basis.

Description. A yellow or light orange, crystalline powder.

Category. Antiacne.

NOTE — It is sensitive to air, heat and light, especially in solution. Carry out all operations as rapidly as possible and avoid exposure to actinic light; use freshly prepared solutions.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isotretinoin IPRS* or with the reference spectrum of isotretinoin.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture 2 volumes of *glacial acetic acid*, 4 volumes of *acetone*, 40 volumes of *peroxide-free ether* and 54 volumes of *cyclohexane.*

Test solution. Dissolve 10 mg of the substance under examination in *dichloromethane* and dilute to 10.0 ml with *dichloromethane.*

Reference solution (a). A 0.1 per cent w/v solution of *isotretinoin IPRS* in *dichloromethane.*

Reference solution (b). A solution containing 0.1 per cent w/v, each of, *isotretinoin IPRS* and *tretinoin IPRS* in *dichloromethane.*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless, the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

C. Dissolve 5 mg in 2 ml of *antimony trichloride solution*; intense red colour develops and later becomes violet.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in *methanol* and dilute to 50.0 ml with *methanol.*

Reference solution (a). A 0.1 per cent w/v solution of *isotretinoin impurity A IPRS* (*tretinoin IPRS*) in *methanol.*

Reference solution (b). Mix 1.0 ml of reference solution (a) with 0.5 ml of the test solution and dilute to 25.0 ml with *methanol.*

Reference solution (c). Dilute 0.5 ml of the test solution to 100.0 ml with *methanol.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 0.5 volume of *glacial acetic acid*, 22.5 volumes of *water* and 77 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 355 nm,
- injection volume: 10 µl.

Name	Relative retention time
Isotretinoin impurity H[1]	0.2
Isotretinoin impurity I[2]	0.3
Isotretinoin (Retention time: about 26 minutes)	
Isotretinoin impurity A[3]	1.34

[1](2*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethyl-3-oxocyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoic acid (13-*cis*-4-oxoretinoic acid),

[2](2*Z*,4*E*,6*E*,8*E*)-9-[(3*RS*)-3-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl]-3,7-dimethylnona-2,4,6,8-tetraenoic acid (13-*cis*-4-hydroxyretinoic acid),

[3](2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (tretinoin).

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to isotretinoin and isotretinoin impurity A is not less than 5.0.

Inject reference solution (c) and the test solution. Run the chromatogram 1.6 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to isotretinoin impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of any peaks corresponding to isotretinoin impurities H and I is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent), the area of any other secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent) and the sum of the areas of all the secondary peaks eluting before the principal peak is not more than 1.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.7 per cent), the sum of areas of all the secondary peaks eluting after the principal peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vaccum for 16 hours.

Assay. Dissolve 0.2 g in 70 ml of *acetone*. Titrate with *0.1 M tetrabutylammonium hydroxide* in *2-propanol*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* in *2-propanol* is equivalent to 0.03004 g of $C_{20}H_{28}O_2$.

Storage. Store protected from light and moisture, under an inert gas.

Labelling. Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

Isotretinoin Capsules

Isotretinoin Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isotretinoin, $C_{20}H_{28}O_2$.

Usual strengths. 5 mg; 10 mg; 20 mg; 30 mg; 40 mg.

Identification

A. In the test for Related substances, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (b).

B. When examined in the range 300 nm to 400 nm (2.4.7), the final solution obtained in the Assay, exhibits a maximum at 356 nm.

Tests

Dissolution (2.5.2).

NOTE — Protect the apparatus from light throughout the determination and flush the apparatus with nitrogen before use.

Apparatus. Used for disintegration test for tablets and capsules (2.5.1),
Medium. 900 ml of *0.1M sodium hydroxide*,
Time. 60 minutes.

Withdraw a suitable volume of the medium and filter immediately through a 0.2 µm filter, discarding the first 5 ml of filtrate, and dilute the filtrate with *0.1 M sodium hydroxide*. Measure the absorbance of the filtrate, suitably diluted if necessary to obtain a solution containing 0.00025 per cent w/v of Isotretinoin, at the maximum at about 343 nm (2.4.7). Calculate the content of $C_{20}H_{28}O_2$ in the medium taking 1490

as the specific absorbance at 343 nm and divide the result by 6 to obtain the amount per capsule.

Q. Not less than 85 per cent of the stated amount of $C_{20}H_{28}O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light.

Test solution (a). Disperse a quantity of the contents of the capsules containing 10 mg of Isotretinoin with 10 ml of a mixture of 20 volumes of *0.1 M sodium hydroxide* and 80 volumes of *ethanol* for 10 minutes with the aid of ultrasound for 15 minutes and centrifuge for 5 minutes. Mix 5 ml of the supernatant liquid with 7.5 ml of *0.1 M hydrochloric acid* and extract with two 20 ml quantities of *dichloromethane*. Filter the combined extracts through phase separating paper (Whatman 1PS is suitable) and wash the filter with 5 ml of *dichloromethane*. Evaporate the combined filtrate and washing to dryness at 30° under reduced pressure and dissolve the residue in 5 ml of *methanol*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with *methanol*.

Reference solution (a). A 0.002 per cent w/v solution of *tretinoin IPRS* in *methanol*.

Reference solution (b). A 0.001 per cent w/v solution of *isotretinoin IPRS* in *methanol*.

Reference solution (c). A solution containing 0.001 per cent w/v, each of, *tretinoin IPRS* and *isotretinoin IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 2),
- mobile phase: a 0.5 per cent w/v solution of *glacial acetic acid* in a mixture of 23 volumes of *water* and 77 volumes of *methanol*,
- flow rate:1.4 ml per minute,
- spectrophotometer set at 353 nm,
- injection volume: 10 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to tretinoin and isotretinoin is not less than 2.0.

Inject reference solution (a), test solution (a) and (b). In the chromatogram obtained with test solution (a), the area of any peak corresponding to tretinoin is not more than twice the area of the peak in the chromatogram obtained with reference solution (a) (4.0 per cent). The area of any other secondary peak is not more than the area of the peak in the chromatogram obtained with test solution (b) (1.0 per cent) and the sum of the areas of any such peaks is not more than 1.5 times the area of the peak in the chromatogram obtained with test solution (b) (1.5 per cent).

Uniformity of content. Complies with the test stated under Capsules.

NOTE — Carry out the test in sub dued light.

Disperse the content of one capsule in 25 ml of *dichloromethane* with the aid of ultrasound for 15 minutes. Add sufficient *dichloromethane* to produce 50 ml, shake for 2 minutes. Dilute a suitable volume of the resulting solution with a solution prepared by diluting 5 ml of *0.1 M hydrochloric acid* to 250 ml with *ethanol (95 per cent)* to produce a solution containing 0.0008 per cent w/v of Isotretinoin. Measure the absorbance of the solution at the maximum at 356 nm (2.4.7), using the *ethanolic hydrochloric acid solution* as blank.

Calculate the content of $C_{20}H_{28}O_2$ in the capsules taking 1350 as the specific absorbance at 356 nm.

Other tests. Comply with the tests stated under Capsules.

Assay. *NOTE — Carry out the test in subdued light.*

Disperse the content of 10 capsules with 25 ml *dichloromethane* per capsule with the aid of ultrasound for 15 minutes. Add sufficient *dichloromethane* to produce 500 ml, shake for 2 minutes. Dilute a suitable volume of the resulting solution with a solution prepared by diluting 5 ml of *0.1 M hydrochloric acid* to 250 ml with *ethanol (95 per cent)* to produce a solution containing 0.0008 per cent w/v of Isotretinoin. Measure the absorbance of the solution at the maximum at 356 nm (2.4.7), using the *ethanolic hydrochloric acid solution* as blank. Calculate the content of $C_{20}H_{28}O_2$ in the Capsules taking 1350 as the specific absorbance at 356 nm.

Storage. Store protected from light.

Isotretinoin Gel

Isotretinoin Gel is a solution of Isotretinoin in a suitable alcoholic basis.

Isotretinoin Gel contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of isotretinoin, $C_{20}H_{28}O_2$.

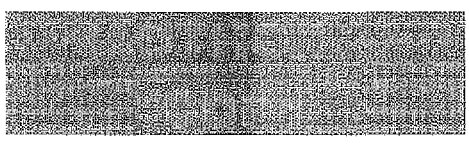
Usual strengths. 0.012 per cent w/w; 0.025 per cent w/w; 0.05 per cent w/w.

NOTE — Carry out all the operations as rapidly as possible and protected from light.

Identification

A. When examined in the range 300nm to 400nm (2.4.7), the solution obtained in the assay, shows an absorption maxima at about 356 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.



Mobile phase. A mixture of 2 volumes of *glacial acetic acid,* 4 volumes of *acetone,* 40 volumes of *peroxide-free ether* and 54 volumes of *cyclohexane.*

Test solution. Shake to disperse a quantity of the gel containing 1 mg of Isotretinoin as completely as possible in a mixture of 5.0 ml of *dichloromethane reagent* and 10.0 ml of *dichloromethane*, dilute to 100 ml with *n-hexane* and shake the mixture vigorously; filter, evaporate the filtrate to dryness using a rotary evaporator at a temperature not exceeding 60° and dissolve the residue in 1.0 ml of *dichloromethane.*

Reference solution. 0.1 per cent w/v solution of *isotretinoin IPRS* in *dichloromethane.*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. After development, remove the plate, dry in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14)

NOTE — Carry out the test protected from light.

Test solution. Shake to disperse a quantity of the gel containing 2.5 mg of Isotretinoin as completely as possible in a mixture of 5.0 ml of *dichloromethane reagent* and 10.0 ml of *dichloromethane*, dilute to 50.0 ml with *n-hexane*, shake and filter.

Reference solution(a). A 0.0002 per cent w/v solution of *tretinoin IPRS* in *n-hexane.*

Reference solution(b). Dilute 1.0 ml of the test solution to 100.0 ml with *n-hexane.*

Reference solution(c). A 0.0002 per cent w/v solution, each of, *isotretinoin IPRS* and *tretinoin IPRS* in *n-hexane.*

Reference solution(d). Dilute 1.0 ml of reference solution (b) to 10.0 ml with *n-hexane.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica gel for chromatography (5 µm) (Lichrosorb Si60 is suitable),
- mobile phase: a mixture of 1 volume of *glacial acetic acid* , 30 volumes of *ethyl acetate* and 970 volumes of *n-hexane,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 365 nm,
- injection volume: 50 µl.

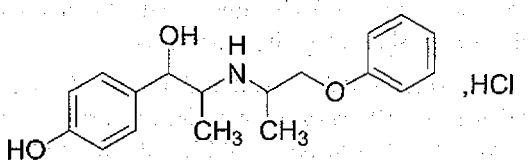
Inject reference solution (c) and (d). The test is not valid unless the resolution between the peaks corresponding to isotretinoin and tretinoin is not less than 3.0 obtained with reference solution (c) and the signal-to-noise ratio of the peak corresponding to isotretinoin is not more than 10 obtained with reference solution (d).

Inject reference solution (a), (b), (d) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to tretinoin is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent) and the sum of the area of all other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Other tests. Comply with the tests stated under Gels.

Assay. To a quantity of the gel containing 0.5 mg of Isotretinoin, add 10 ml of *dichloromethane*, shake until all the gel has dispersed and dilute the solution to 100.0 ml with *ethanolic hydrochloric acid solution* prepared by diluting 5 ml of *0.1M hydrochloric acid* to 250 ml with *ethanol (95 per cent).* Measure the absorbance of the resulting solution at the maximum at 356 nm, using the *ethanolic hydrochloric acid solution* as blank (2.4.7). Calculate the content of $C_{20}H_{28}O_2$ in the gel taking 1350 as specific absorbance at the maximum at 356 nm.

Isoxsuprine Hydrochloride



$C_{18}H_{23}NO_3,HCl$ Mol. Wt. 337.8

Isoxsuprine Hydrochloride is (1*RS*,2*SR*)-1-(4-hydroxy-phenyl)-2-[(1*RS*)-1-methyl-2-phenoxyethylamino]propan-1-ol hydrochloride.

Isoxsuprine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{23}NO_3,HCl$, calculated on the dried basis.

Category. Vasodilator; uterine relaxant.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoxsuprine hydrochloride IPRS* or with the reference spectrum of isoxsuprine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.1 M hydrochloric acid* shows absorption maxima at about 269 nm and 274 nm; absorbance at about 269 nm, about 0.73 and at about 274 nm, about 0.72.

C. Dissolve 10 mg in 1 ml of *water* and add 0.05 ml of *copper sulphate solution* and 1 ml of *5 M sodium hydroxide*; a blue colour is produced. Add 1 ml of *ether* and shake; the ether layer remains colourless.

D. It gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution, prepared with gentle warming if necessary.

Appearence of solution. A 1.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

Optical rotation (2.4.22). – 0.05° to + 0.05°, determined in a 1.0 per cent w/v solution, prepared with gentle warming, if necessary.

Related substances. Determine by gas chromatography (2.4.13).

NOTE— Prepare the solutions immediately before use.

Internal standard solution (a). Dissolve 0.1 g of *hexacosane* in 20.0 ml of *trimethylpentane.*

Internal standard solution (b). Dilute 1.0 ml of internal standard solution (a) to 50.0 ml with *trimethylpentane.*

Test solution. Add 0.5 ml of *N-trimethylsilylimidazole* to 10 mg of the substance under examination. Heat to 65° for 10 minutes, cool. Add 2.0 ml of internal standard solution (b) and 2.0 ml of *water*, shake. Use the upper layer.

Reference solution (a). Add 0.5 ml of *N-trimethylsilylimidazole* to 10 mg of the substance under examination. Heat to 65° for 10 minutes, cool. Add 2.0 ml of internal standard solution (a) and 2.0 ml of *water*, shake. Dilute 1.0 ml of the upper layer to 50.0 ml with *trimethylpentane.*

Reference solution (b). Add 0.5 ml of *N-trimethylsilylimidazole* to 10 mg of the substance under examination. Heat to 65° for 10.0 min, cool. Add 2.0 ml of *trimethylpentane* and 2.0 ml of *water*, shake. Use the upper layer.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with diatomaceous support (125 to 135 µm) coated with 3 per cent w/w of poly(dimethyl)siloxane,
- temperature:

Column	time (min)	temperature (°)
	0-25	195
	25-29	195-215
	29-39	215

- inlet and detector port 225°,
- flame ionization detector,
- flow rate: 30 ml per minute, nitrogen as the carrier gas.

Inject 1 µl of the test solution and reference solution (a). The test is not valid unless the resolution between the peaks due to isoxsurpine and hexacosane is not less than 5.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to the trimethylsilyl derivative of isoxsuprine to the area of the peak due to hexacosane from the chromatogram obtained with reference solution (a). The ratio of the sum of the areas of peaks, other than the principal peak and the peak due to hexacosane to the area of the peak due to the hexacosane is not more than 2.0 per cent.

Phenones. Absorbance of a 0.01 per cent w/v solution at about 310 nm, not more than 0.20 (2.4.7).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.6 g, dissolve in 150 ml of *anhydrous glacial acetic acid*, heating on a water-bath to effect dissolution. Cool and add 15 ml of *mercuric acetate solution.* Titrate with *0.1 M perchloric acid*, using *naphtholbenzein* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03378 g of $C_{18}H_{23}NO_3$,HCl.

Storage. Store protected from light.

Isoxsuprine Injection

Isoxsuprine Hydrochloride Injection

Isoxsuprine Injection is a sterile solution of Isoxsuprine Hydrochloride in Water for Injections.

Isoxsuprine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of isoxsuprine hydrochloride, $C_{18}H_{23}NO_3$,HCl.

Usual strength. 5 mg per ml.

Identification

A. To a volume containing 50 mg of Isoxsuprine Hydrochloride add 20 ml of *water* and 10 ml of *ammonia buffer pH 10.0* and extract with three quantities, each of 15 ml, of *dichloromethane.* Shake the combined extracts with 5 g of *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness, dissolve the residue in 5 ml of *0.1 M methanolic hydrochloric acid* and

evaporate to dryness. Dissolve the residue in 5 ml of *methanol*, evaporate to dryness, redissolve the residue in 2 ml of *methanol*, add 15 ml of *dichloromethane*, again evaporate to dryness and dry the residue at 60° at a pressure of 2 kPa for 1 hour. On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoxsuprine hydrochloride IPRS* treated in the same manner or with the reference spectrum of isoxsuprine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 269 nm and 274 nm.

Tests

pH (2.4.24). 4.9 to 6.0.

Bacterial endotoxins (2.2.3). Not more than 35.7 Endotoxin Units per mg of isoxsuprine hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume containing about 50 mg of Isoxsuprine Hydrochloride add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml. Dilute 10.0 ml to 100.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 274 nm (2.4.7). Calculate the content of $C_{18}H_{23}NO_3$,HCl taking 73 as the specific absorbance at 274 nm.

Isoxsuprine Tablets

Isoxsuprine Hydrochloride Tablets

Isoxsuprine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of isoxsuprine hydrochloride, $C_{18}H_{23}NO_3$,HCl.

Usual strength. 20 mg.

Identification

A. To a quantity of the powdered tablets containing 50 mg of Isoxsuprine Hydrochloride add 50 ml of *0.1 M hydrochloric acid* and heat on a water-bath for 30 minutes. Cool, filter, add 10 ml of *ammonia buffer pH 10.0* and extract with three quantities, each of 15 ml, of *dichloromethane*. Shake the combined extracts with 5 g of *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness, dissolve the residue in 5 ml of *0.1 M methanolic hydrochloric acid* and evaporate to dryness. Dissolve the residue in 5 ml of *methanol*, evaporate to dryness, redissolve the residue in 2 ml of *methanol*, add 15 ml of *dichloromethane*, again evaporate to dryness and dry the residue at 60° at a pressure of 2 kPa for 1 hour. On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *isoxsuprine hydrochloride IPRS* treated in the same manner or with the reference spectrum of isoxsuprine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 269 nm and 274 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium: 900 ml of *water*,
Speed and time. 100 rpm for 45 minutes.

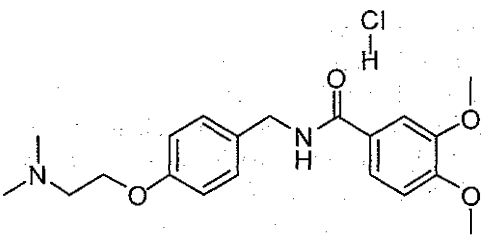
Withdraw a suitable volume of the medium and filter, rejecting the first few ml of filtrate. Dilute a suitable volume of the filtrate with the medium, if necessary. Measure the absorbance of the resulting solution at the maximum at about 269 nm (2.4.7). Calculate the content of isoxsuprine hydrochloride, $C_{18}H_{23}NO_3$, HCl in the medium from the absorbance obtained from a solution of known concentration of *isoxsuprine hydrochloride IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{18}H_{23}NO_3$, HCl.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 20 mg of Isoxsuprine Hydrochloride, add 50 ml of *0.1 M hydrochloric acid* and boil on a water-bath for 30 minutes. Cool, add sufficient *0.1 M hydrochloric acid* to produce 100 0 ml, mix and filter. Dilute 25.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 274 nm (2.4.7). Calculate the content of $C_{18}H_{23}NO_3$,HCl taking 73 as the specific absorbance at 274 nm.

Itopride Hydrochloride



$C_{20}H_{26}N_2O_4$, HCl Mol. Wt. 394.9

Itopride Hydrochloride is N-[[4-[2-(dimethylamino)ethoxy] phenyl]methyl]-3,4-dimethoxybenzamide; hydrochloride.

Itopride Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent $C_{20}H_{26}N_2O_4$, HCl, calculated on the dried basis.

Category. Gastroprokinetic Agent, Propulsives.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *itopride hydrochloride IPRS* or with the reference spectrum of itopride hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 5.5, determined in a 10 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of the areas of all secondary peaks is not more than 1.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the same solvent.

Reference solution. A 0.025 per cent w/v solution of *itopride hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 55 volumes of *0.05 M citric acid*, adjusted to pH 3.0 with *sodium hydroxide solution* and 45 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 258 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{26}N_2O_4$, HCl.

Itopride Tablets

Itopride Hydrochloride Tablets

Itopride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of itopride hydrochloride, $C_{20}H_{26}N_2O_4$, HCl.

Usual strength. 50 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute a suitable volume of the filtrate with the dissolution medium, if necessary, measure the absorbance of the resulting solution at the maxima at about 257 nm (2.4.7). Calculate the content of $C_{20}H_{26}N_2O_4$, HCl in the medium from the absorbance obtained from a solution of known concentration of *itopride hydrochloride IPRS* in the same medium.

Q. Not less than 70 per cent of the stated amount of $C_{20}H_{26}N_2O_4$, HCl.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse 5 intact tablets in 300 ml of the solvent mixture with the aid of ultrasound for about 15 minutes and dilute to 500.0 ml with the solvent mixture and filter.

Inject the test solution. The area of any secondary peak is not more than 1.0 per cent and the sum of the areas of all secondary peaks is not more than 2.0 per cent, calculated by area normalisation.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *water* and 30 volumes of *methanol*.

Test solution. Disperse 5 intact tablets in 300 ml of the solvent mixture with the aid of ultrasound for about 15 minutes and dilute to 500.0 ml with the solvent mixture and filter. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A 0.0025 per cent w/v solution of *itopride hydrochloride IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *0.05 M citric acid*, adjusted to pH 3.0 with *sodium hydroxide solution* and 45 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 258 nm,
- injection volume: 20 µl.

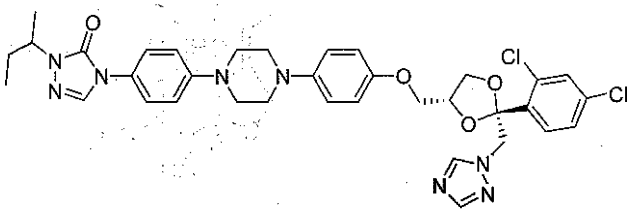
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{26}N_2O_4$, HCl in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Itraconazole



$C_{35}H_{38}Cl_2N_8O_4$ Mol. Wt. 706.0

Itraconazole is 4-[4-[4-[4-[[*cis*-2-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one.

Itraconazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{35}H_{38}Cl_2N_8O_4$, calculated on the dried basis.

Category. Antifungal

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *itraconazole IPRS* or with the reference spectrum of itraconazole.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *dichloromethane* is clear (2.4.1) and not more intensely coloured than reference solution RS6 or BS6 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 0.1 g of the substance under examination in *methanolic hydrochloric acid* and dilute to 10.0 ml with *methanolic hydrochloric acid.*

Reference solution (a). A 0.001 per cent w/v solution of *itraconazole IPRS* in *methanolic hydrochloric acid.*

Reference solution (b). Dissolve 10 mg of *itraconazole system suitability IPRS* (containing impurities B, C, D, E, F and G) in 1.0 ml of *methanolic hydrochloric acid.*

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with base deactivated end-capped octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a 2.72 per cent w/v solution of *tetrabutylammonium hydrogen sulphate* in *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
2	80	20
22	50	50
27	50	50
30	80	20

Name	Relative retention time
Itraconazole impurity B[1]	0.7
Itraconazole impurity C[2] and D[3]	0.8
Itraconazole impurity E[4]	0.9
Itraconazole (retention time: about 14 minutes)	1.0
Itraconazole impurity F[5]	1.05
Itraconazole impurity G[6]	1.3

[1]4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(4*H*-1,2,4-triazol-4-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(1*RS*)-1-methylpropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one,

[2]4-[4-[4-[4-[[*cis*-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-propyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one,

[3]4-[4-[4-[4-[[cis-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-(1-methylethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one,

[4]4-[4-[4-[4-[[trans-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(1RS)-1-methylpropyl]-2,4-dihydro-3H-1,2,4-triazol- 3-one,

[5]2-butyl-4-[4-[4-[4-[[cis-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2,4-dihydro-3H-1,2,4-triazol-3-one,

[6]4-[4-[4-[4-[[cis-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[[cis 2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl]-2,4-dihydro-3H-1,2,4-triazol-3-one.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 1.5, where Hp is the height above the baseline of the peak due to itraconazole impurity F and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to itraconazole.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to itraconazole impurity B and impurity G, each of, is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any peak corresponding to itraconazole impurity E is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the sum of areas of the peaks corresponding to itraconazole impurity C and impurity D is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1g by drying in an oven at 105° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of substance under examination in 50 ml of *methanolic hydrochloric acid* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with *methanolic hydrochloric acid*.

Reference solution. A 0.01 per cent w/v solution of *itraconazole IPRS* in *methanolic hydrochloric acid*. Use Chromatographic system as described under Related substances.

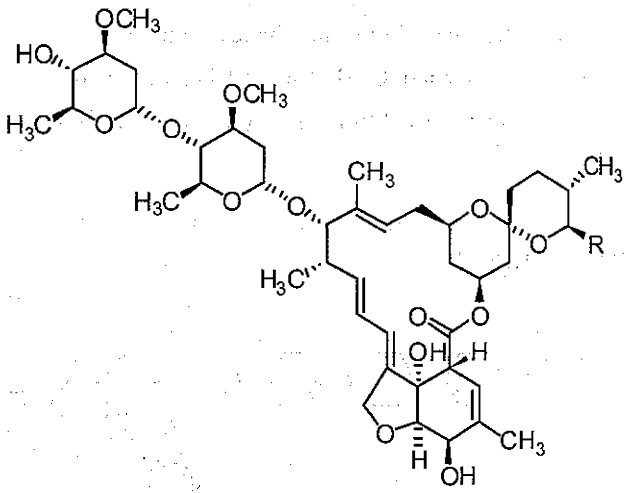
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{35}H_{38}Cl_2N_8O_4$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Ivermectin



$C_{48}H_{74}O_{14}$, H_2B_{1a} Mol.Wt. 875.1

$C_{47}H_{72}O_{14}$, H_2B_{1b} Mol.Wt. 861.1

Ivermectin is (10*E*,14*E*,16*E*,22*Z*)-(1*R*,4*S*,5'*S*,6*R*,6'*R*,8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)-6'-[alkyl]-21,24-dihydroxy-5',11,13,22-tetramethyl-2-oxo-(3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentocsa-10,14,16,22-tetraene)-6-spiro-2'-(perhydropyran)-12-yl-2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-*L*-arabino-hexopyranosy)-3-*O*-methyl-α-*L*-arabinohexopyranoside.

Ivermectin contains not less than 95.0 per cent and not more than 102.0 per cent of H_2B_{1a}+ H_2B_{1b}, calculated on the anhydrous and solvent free basis.

The ratio $H_2B_{1a}/(H_2B_{1a}+ H_2B_{1b})$, determined by liquid chromatography is not less than 90.0 per cent.

Category.Anthelmintic.

Description. A white or yellowish-white crystalline powder, slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometery (2.4.6). Compare the spectrum with that obtained with *ivermectin IPRS* or with the reference spectrum of ivermectin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *toluene* is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

Specific optical rotation (2.4.22). –20.0° to –17.0°, determined on a 2.5 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in *methanol* and dilute to 50.0 ml with *methanol*.

Reference solution (a). A 0.08 per cent w/v solution of *ivermectin IPRS* in *methanol*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol*.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 100.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 15 volumes of *water*, 34 volumes of *methanol* and 51 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (a) and (c). The test is not valid unless in the chromatogram obtained with reference solution (a), the resolution between the component H_2B_{1b} (first peak) and component H_2B_{1a} (second peak) is not less than 3.0 and in the chromatogram obtained with reference solution (c), the signal to noise ratio is not less than 10 for the principal peak.

Inject the test solution and reference solution (b) and (c). In the chromatogram obtained with the test solution the impurity with a relative retention of 1.3 to 1.5 with reference to the principal peak is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Ethanol and formamide. Not more than 5.0 per cent ethanol and not more than 3.0 per cent formamide.

Determine by gas chromatography (2.4.13).

Internal standard solution. Dilute 0.5 ml of *propanol* to 100.0 ml with *water*.

Test solution. Dissolve 0.120 g of the substance under examination in 2.0 ml of *m-xylene* by heating on a water-bath at 40 to 50°, add 2.0 ml of *water*, mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 ml of *water*. Discard the upper layer and combine the aqueous layers. Add 1.0 ml of the internal standard solution. Centrifuge and discard any remaining *m-xylene*.

Reference solution (a). Dilute 3.0 g of *ethanol* to 100 ml with *water*.

Reference solution (b). Dilute 1.0 g of *formamide* to 100.0 ml with *water*.

Reference solution (c). Dilute 5.0 ml of reference solution (a) and 5 ml of reference solution (b) to 50.0 ml with *water*. Transfer 2.0 ml of the solution to a centrifuge tube, add 2 ml of *m-xylene*, mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 ml of *water*. Discard the upper layer and combine the aqueous layers. Add 1.0 ml of the internal standard solution. Centrifuge and discard any remaining *m-xylene*.

Reference solution (d). Dilute 10.0 ml of reference solution (a) and 10.0 ml of reference solution (b) to 50.0 ml with *water*. Transfer 2.0 ml of the solution to a centrifuge tube, add 2 ml of *m-xylene*, mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 ml of *water*. Discard the upper layer and combine the aqueous layers. Add 1.0 ml of the internal standard solution. Centrifuge and discard any remaining *m-xylene*.

Chromatographic system
- a capillary column 30 m x 0.53 mm, packed with fused silica with macrogol 20,000 with film thickness 1 µm,
- temperature

column	time (min)	temperature (°)
	0-2	50 → 80
	2-8	80 → 240

- injection port 220° and detector at 280°,
- flame ionization detector,
- flow rate: 7.5 ml per minute using nitrogen as the carrier gas.

Inject 1 µl of reference solution (c), (d) and the test solution.

Calculate the content of *ethanol* and *formamide*.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method C (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography(2.4.14), as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the contents of ivermectin (H_2B_{1a} + H_2B_{1b}) and the ratio $H_2B_{1a}/(H_2B_{1a}+H_2B_{1b})$.

Storage. Store protected from moisture.

Ivermectin Injection

Ivermectin Injection is a sterile solution of Ivermectin in a suitable non-aqueous vehicle.

Ivermectin contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sum of H_2B_{1a} ($C_{48}H_{74}O_{14}$) and H_2B_{1b}($C_{47}H_{72}O_{14}$).

The ratio of $H_2B_{1a}/(H_2B_{1a}+H_2B_{1b})$ is not less than 90.0 per cent.

Usual strength. 10 mg per ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *ammonia*, 9 volumes of *methanol* and 90 volumes of *dichloromethane*.

Test solution. Dissolve a volume of the injection in *methanol* to obtain a solution containing 0.05 per cent w/v of Ivermectin.

Reference solution. A 0.05 per cent w/v solution of *ivermectin IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the two principal peaks in the chromatogram obtained with the test solution corresponds to the principal peaks in the chromatogram obtained with reference solution.

Tests

Appearance of solution. The injection is clear (2.4.1) and not more intensely coloured than reference solution YS4 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a volume of injection in *methanol* to obtain a solution containing 0.04 per cent w/v of Ivermectin.

Reference solution(a). A 0.04 per cent w/v solution of *ivermectin IPRS* in *methanol*.

Reference solution (b). A 0.0004 per cent w/v solution of *ivermectin IPRS* in *methanol*.

Reference solution (c). A 0.00002 per cent w/v solution of *ivermectin IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 39 volumes of *water*, 55 volumes of *methanol* and 106 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless in the chromatogram obtained with reference solution (a), the resolution between the component H_2B_{1b} (first peak) and component H_2B_{1a} (second peak) is not less than 3.0.

Inject reference solution (b), (c) and the test solution. In the chromatogram obtained with the test solution the area of any peak with a relative retention of 1.3 to 1.5 with reference to the principal peak is not more than 2.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.7 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (6.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.016 Endotoxin Unit per µg of ivermectin.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a volume of the injection in *methanol* to obtain a solution containing 0.04 per cent w/v of Ivermectin.

Reference solution. A 0.04 per cent w/v solution of *ivermectin IPRS* in *methanol*.

Use chromatographic conditions as described under Related substances.

Inject the reference solution and the test solution.

Calculate the content of ivermectin (H_2B_{1a} + H_2B_{1b}) in the injection and the ratio H_2B_{1a} / (H_2B_{1a} + H_2B_{1b}).

Ivermectin Tablets

Ivermectin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ivermectin calculated as sum of ivermectin components H_2B_{1a} ($C_{48}H_{74}O_{14}$) and H_2B_{1b} ($C_{47}H_{72}O_{14}$). This may contain suitable antioxidants.

Usual strengths. 3 mg; 6 mg; 12 mg.

Identification

In the Assay, the retention times of the H_2B_{1a} and H_2B_{1b} peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a buffer solution prepared by dissolving 5 g of *sodium dodecyl sulphate* in 900 ml of *water*, add 10 ml of *1 M monobasic sodium phosphate monohydrate*, adjusted to pH 7.0 with *sodium hydroxide solution,* and dilute to 1000 ml with *water.*

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a weighed quantity of *ivermectn IPRS* in minimum amount of *methanol* and dilute with the dissolution medium to obtain a solution of similar concentration as that of the test solution.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 53 volumes of *acetonitrile*, 35 volumes of *methanol* and 12 volumes *water,*
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume:100 µl.

The relative retention time with reference to H_2B_{1a} for H_2B_{1b} is about 0.8.

Inject the reference solution. The test is not valid unless the resolution between H_2B_{1a} and H_2B_{1b} peaks is not less than1.5, the capacity factor for the H_2B_{1a} peak is not less than 4.0, column efficiency determine from both H_2B_{1a} and H_2B_{1b} peaks is not less than 1500 theoretical plates, the tailing factor for the H_2B_{1a} is not more than 2.0 and the relative standard deviation for replicate injections for the H_2B_{1a} is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the combined content of H_2B_{1a} and H_2B_{1b} in the medium.

Q. Not less than 80 per cent of the stated amount of ivermectin calculated as sum of H_2B_{1a} ($C_{48}H_{74}O_{14}$) and H_2B_{1b} ($C_{47}H_{72}O_{14}$) in the medium.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Disperse one tablet in 5 ml of *water* with the aid of ultrasound for 10 minutes. Add 15 ml of *methanol,* sonicate for 5 minutes and mix. Allow the solution cool to room temperature and dilute to 25.0 ml with *methanol.*

Reference solution. Dissolve a weighed quantity of *ivermectn IPRS* with *methanol* to obtain a solution of similar concentration as that of the test solution.

Inject the reference solution and the test solution.

Calculate the content of ivermectin calculated as sum of H_2B_{1a} ($C_{48}H_{74}O_{14}$) and H_2B_{1b} ($C_{47}H_{72}O_{14}$) in the tablets.

Limit of 8a-oxo- H_2B_{1a}. Not more than 2 per cent.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

- spectrophotometer set at 280 nm,

Reference solution. A 0.000096 per cent w/v solution of *3-tert-Butyl-4-hydroxyanisole (BHA) IPRS* in *methanol.*

The relative retention time with reference to H_2B_{1a} for BHA and 8a-oxo- H_2B_{1a} are about 0.24 and 0.77 respectively.

Inject the reference solution and the test solution.

Calculate the percentage of 8a-oxo-H_2B_{1a} as a percentage of the label claim of ivermectin in the portion of Tablets by using the following formula

$$\frac{r_u}{r_s} \times \frac{W_S}{W_U} \times \frac{D_U}{D_S} \times \frac{C_F}{f} \times \frac{P}{L} \times 100$$

in which r_u and r_s is the peak area of 8a-oxo-H_2B_{1a} obtained from the test solution and peak area of BHA obtained from the reference solution, W_S is the weight in mg of *BHA IPRS*, P is the purity of *BHA IPRS,* expressed as a decimal, D_U and D_S is dilution factor of test solution and reference solution, C_F is

the correction factor (equal to 0. 98) used to convert mg of 8a-oxo-H_2B_{1a} to mg of ivermectin, W_U is the number of tablets taken to prepare the test solution, L is the label claim in mg per tablet of ivermectin and 'f' is the correction factor (equal to 1.0).

$$\text{Correction factor (CF)} = \frac{0.90\ (\text{molecular weight of } H_2B_{1a}) + 0.10\ (\text{molecular weight of } H_2B_{1b})}{\text{molecular weight of } 8a - \text{oxo} - H_2B_{1a}}$$

$$CF = \frac{873.10}{889.10} = 0.98$$

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 60 mg of Ivermectin in 25 ml of *water,* with the aid of ultrasound for 10 minutes and dilute to 250.0 ml with *methanol.*

Reference solution (a). A 0.025 per cent w/v solution of *ivermectin IPRS* in *methanol.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol.* Dilute 1.0 ml of the solution to 5.0 ml with *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 53 volumes of *acetonitrile,* 35 volumes of *methanol* and 12 volumes *water,*
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume:10 µl.

The relative retention time with reference to H_2B_{1a} for H_2B_{1b} is about 0.8.

Inject reference solution (a) and (b). The test is not valid unless the column efficiency for H_2B_{1a} is not less than 1500 theoretical plates, the capacity factor for H_2B_{1b} is not less than 3.0, the tailing factor for H_2B_{1a} is not more than 2.0 and relative standard deviation for the area response of total ivermectin (H_2B_{1a} and H_2B_{1b}) for replicate injections is not more than 2.0 per cent in reference solution (a) and the signal-to-noise ratio of the principal peak is not less than 10.0 in reference solution (b).

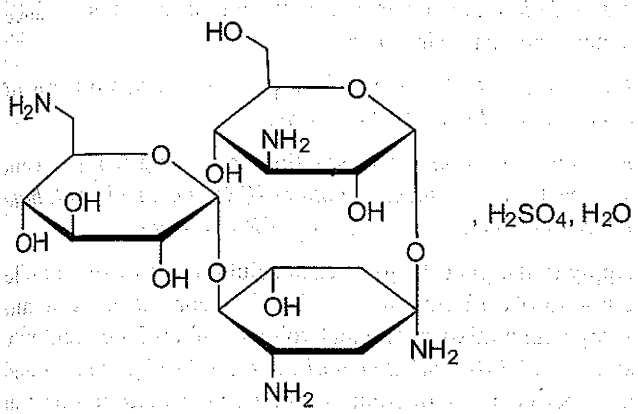
Inject reference solution (a) and the test solution, measure sum of the peak areas for content H_2B_{1a} and H_2B_{1b}.

Calculate the content of ivermectin calculated as sum of H_2B_{1a} ($C_{48}H_{74}O_{14}$) and H_2B_{1b} ($C_{47}H_{72}O_{14}$) in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

K

Kanamycin Sulphate



, H_2SO_4, H_2O

$C_{18}H_{36}N_4O_{11},H_2SO_4,H_2O$ Mol. Wt. 600.6

Kanamycin Sulphate is 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxy-D-streptamine sulphate monohydrate, an antimicrobial substance produced by the growth of certain strains of *Streptomyces kanamyceticus*.

Kanamycin Sulphate has a potency of not less than 750 Units per mg, calculated on the dried basis.

Category. Antibacterial.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjusted to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7.0 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. A 0.1 per cent w/v solution of the substance under examination in *water*.

Reference solution (a). A 0.1 per cent w/v solution of *kanamycin sulphate IPRS* in *water*.

Reference solution (b). A solution containing 0.1 per cent w/v, each of, *kanamycin sulphate IPRS*, *neomycin sulphate IPRS* and *streptomycin sulphate IPRS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *1,3-napthalenediol* in *ethanol (95 per cent)* and a 45 per cent w/v solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 0.5 g in 10 ml of *water* and add 10 ml of a 1 per cent w/v solution of *picric acid*. If necessary initiate crystallisation by scratching the walls of the container with a glass rod, allow to stand and filter. The crystals, after washing with 20 ml of *water* and drying at 105°, melt at about 235°, with decomposition (2.4.21).

C. Dissolve 50 mg in 2 ml of *water*, add 1 ml of a 1 per cent w/v solution of *ninhydrin* and heat for a few minutes on a water-bath; a violet colour is produced.

D. It gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 6.5 to 8.5, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). +112° to +123°, determined at 20° in a 1.0 per cent w/v solution.

Kanamycin B. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjusted to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. A 0.5 per cent w/v solution of the substance under examination in *water*.

Reference solution. A 0.02 per cent w/v solution of *kanamycin B sulphate IPRS* in *water*.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphates. 15.0 to 17.0 per cent of SO_4, calculated on the dried basis and determined by the following method. Dissolve 0.25 g in 100 ml of *water* and adjusted to pH 11 using *strong ammonia solution*. Add 10.0 ml of *0.1 M barium chloride* and 0.5 mg of *metalphthalein*. Titrate with *0.1 M disodium edetate*; when the colour of the solution begins to change,

add 50 ml of *ethanol (95 per cent)* and continue the titration until the blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of SO_4.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

Kanamycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

Kanamycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture. If the contents are intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) whether or not the material is intended for use in the manufacture of parenteral preparations.

Kanamycin Acid Sulphate

Kanamycin Acid Sulphate is a form of kanamycin sulphate prepared by adding Sulphuric Acid to a solution of Kanamycin Sulphate and subsequent drying.

Kanamycin Acid Sulphate has a potency of not less than 650 Units per mg, calculated on the dried basis.

Category. Antibacterial.

Description. A white or almost white powder; hygroscopic.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjusted to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. A 0.1 per cent w/v solution of the substance under examination in *water*.

Reference solution (a). A 0.1 per cent w/v solution of *kanamycin sulphate IPRS* in *water*.

Reference solution (b). A solution containing 0.1 per cent w/v, each of, *kanamycin sulphate IPRS*, *neomycin sulphate IPRS* and *streptomycin sulphate IPRS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *1,3-napthalenediol* in *ethanol (95 per cent)* and a 45 per cent w/v of solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 0.5 g in 10 ml of *water* and add 10 ml of a 1 per cent w/v solution of *picric acid*. If necessary initiate crystallisation by scratching the walls of the container with a glass rod, allow to stand and filter. The melting point of the crystals, after washing with 20 ml of *water* and drying at 105°, is about 235°, with decomposition (2.4.21).

C. Dissolve 50 mg in 2 ml of *water*, add 1 ml of a 1 per cent w/v solution of *ninhydrin* and heat for a few minutes on a water-bath; a violet colour is produced.

D. It gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 5.5 to 7.5, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). +103° to +115°, determined at 20° in a 1.0 per cent w/v solution.

Kanamycin B. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjusted to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. A 0.5 per cent w/v solution of the substance under examination in *water*.

Reference solution. A 0.02 per cent w/v solution of *kanamycin B sulphate IPRS* in *water*.

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphates. 23.0 to 26.0 per cent of SO_4, calculated on the dried basis and determined by the following method. Dissolve 0.25 g in 100 ml of *water* and adjusted to pH 11 using *strong ammonia solution*. Add 10.0 ml of *0.1 M barium chloride* and 0.5 mg of *metalphthalein*. Titrate with *0.1 M disodium edetate*; when the colour of the solution begins to change, add 50 ml of *ethanol (95 per cent)* and continue the titration until the blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of SO_4.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

Kanamycin Acid Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

Kanamycin Acid Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture. If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) whether or not the material is intended for use in the manufacture of parenteral preparations.

Kanamycin Injection

Kanamycin Injection is either a sterile solution of Kanamycin Sulphate in Water for Injections containing Sulphuric Acid and suitable buffering and stabilising agents or, is a sterile material consisting of Kanamycin Acid Sulphate with buffering agents and other excipients. It is filled in a sealed container.

A. *Kanamycin Injection (Solution)*

Kanamycin Injection contains Kanamycin Sulphate equivalent to not less than 97.0 per cent and not more than 110.0 per cent of the stated number of Units of kanamycin.

Usual strengths. The equivalent of 250 mg (250,000 Units) of kanamycin per ml or per sealed container.

Description. A colourless to pale yellow solution.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjusted to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. A suitable volume diluted with *water* to contain 800 Units per ml.

Reference solution (a). A 0.1 per cent w/v solution of *kanamycin sulphate IPRS* in *water*.

Reference solution (b). A solution containing 0.1 per cent w/v, each of, *kanamycin sulphate IPRS*, *neomycin sulphate IPRS* and *streptomycin sulphate IPRS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *1,3-napthalenediol* in *ethanol (95 per cent)* and a 45 per cent w/v of solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

Tests

pH (2.4.24). 4.0 to 6.0.

Kanamycin B. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjusted to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 130 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7.0 per cent w/v solution of *potassium dihydrogen phosphate.*

Test solution. A suitable volume diluted with *water* to contain 3750 Units per ml.

Reference solution. A 0.02 per cent w/v solution of *kanamycin B sulphate IPRS* in *water.*

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with *ninhydrin* and *stannous chloride reagent* and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

The upper fiducial limit of error is not less than 97.0 per cent and the lower fiducial limit of error is not more than 110.0 per cent of the stated number of Units.

B. *Kanamycin Injection (Powder)*

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Kanamycin Injection contains not less than 95.0 per cent and not more than 115.0 per cent of the stated number of Units of kanamycin

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjusted to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H.* Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7.0 per cent w/v solution of *potassium dihydrogen phosphate.*

Test solution. A suitable volume diluted with *water* to contain 800 Units per ml.

Reference solution (a). A 0.1 per cent w/v solution of *kanamycin sulphate IPRS* in *water.*

Reference solution (b). A solution containing 0.1 per cent w/v, each of, *kanamycin sulphate IPRS, neomycin sulphate IPRS* and *streptomycin sulphate IPRS* in *water.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *1,3-napthalenediol* in *ethanol (95 per cent)* and a 45 per cent w/v of solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

Tests

pH (2.4.24). 5.5 to 7.5, determined in a 1.0 per cent w/v solution.

Kanamycin B. Determine by thin-layer chromatography (2.4.17), coating the plate with a 0.75-mm layer of the following mixture. Mix 0.3 g of *carbomer* (Such as Carbopol 934) with 240 ml of *water* and allow to stand, with moderate shaking, for 1 hour; adjusted to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H.* Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of *potassium dihydrogen phosphate.*

Test solution. A suitable volume diluted with *water* to contain 3750 Units per ml.

Reference solution. A 0.02 per cent w/v solution of *kanamycin B sulphate IPRS* in *water.*

Apply to the plate 4 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Any spot corresponding to kanamycin B in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Bacterial endotoxins (2.2.3). Not more than 0.67 Endotoxin Unit per mg of kanamycin.

Assay. Determine the weight of the contents of 10 containers. Using the mixed contents of the 10 containers determine by

the microbiological assay of antibiotics, Method A or Method B (2.2.10).

For a container of average content weight, the upper fiducial limit of error is not less than 95.0 per cent and the lower fiducial limit of error is not more than 115.0 per cent of the stated number of Units.

Storage. Store in single dose containers protected from light and moisture.

Labelling. The label states (1) the strength in terms of the number of Units or the equivalent weight of kanamycin in a suitable dose-volume or in the sealed container; (2) the volume of Water for Injections for constituting the solution (for contents of a sealed container).

Heavy Kaolin

Heavy Kaolin is a purified, natural, hydrated aluminium silicate of variable composition.

Category. Pharmaceutical aid.

Description. Fine, white or greyish white, soft powder.

Identification

A. To 0.5 g in a metal crucible add 1 g of *potassium nitrate* and 3 g of *anhydrous sodium carbonate*, heat until the mixture has melted and allow to cool. To the residue add 20 ml of boiling *water*, mix, filter and wash the residue with 50 ml of *water*. To the residue add 1 ml of *hydrochloric acid* and 5 ml of *water* and filter. To the filtrate add 1 ml of *10 M sodium hydroxide* and filter. To the filtrate add 3 ml of *ammonium chloride solution*; a gelatinous, white precipitate is produced.

B. 0.25 g gives the reaction of silicates (2.3.1).

Tests

Acidity or alkalinity. To 1.0 g add 20 ml of *carbon dioxide-free water*, shake for 2 minutes and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.25 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to pink.

Arsenic (2.3.10). Disperse 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Boil 5.0 g with 7.5 ml of *2 M hydrochloric acid* and 27.5 ml of *water* for 5 minutes, filter, wash the residue with *water* and dilute the combined filtrate and washings to 50 ml with *water* (solution A). To 5 ml of solution A add 5 ml of *water*, 10 ml of *hydrochloric acid* and 25 ml of *4-methylpentan-2-one*, shake for 2 minutes, allow the layers to separate and evaporate the aqueous layer to dryness on a water-bath. Dissolve the residue in 1 ml of *5 M acetic acid*, dilute to 25 ml with *water* and filter. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (50 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Chlorides (2.3.12). Shake 4.0 g with a mixture of 34 ml of *distilled water* and 6 ml of *5 M acetic acid* for 1 minute and filter. 10 ml of the filtrate complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). 2 ml of the filtrate obtained in the test for chlorides diluted to 15 ml complies with the limit test for sulphates (750 ppm).

Substances soluble in mineral acids. Not more than 1 per cent, determined by the following method. To 10 ml of solution A add 1.5 ml of *1 M sulphuric acid*, evaporate to dryness on a water-bath, ignite, cool and weigh.

Organic impurities. Heat 0.3 g to redness in a calcination tube. The residue is only slightly more coloured than the original substance.

Adsorption power. In a ground-glass-stoppered test-tube shake 1.0 g with 10 ml of a 0.37 per cent w/v solution of *methylene blue* for 2 minutes and allow to settle. Centrifuge and dilute 1 volume of the solution to 100 volumes with *water*. The solution is not more intensely coloured than a 0.003 per cent w/v solution of *methylene blue*.

Swelling power. Triturate 2 g with 2 ml of *water*; the mixture does not flow.

Loss on ignition (2.4.20). Not more than 15.0 per cent, determined on 1.0 g.

Light Kaolin

Light Kaolin is a native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried. It may contain a suitable dispersing agent.

Category. Pharmaceutical aid.

Description. Light, white powder free from gritty particles; unctuous to the touch.

Identification

A. Fuse 1 g with 2 g of *anhydrous sodium carbonate*, warm the residue with 10 ml of *water*, filter, wash the filter with 5 ml of *water* and reserve the residue. To the combined filtrate and washings add 3 ml of *hydrochloric acid*; a gelatinous precipitate is produced.

B. Dissolve the residue reserved in test A in 10 ml of *2 M hydrochloric acid*; the solution gives reaction (B) of aluminium salts (2.3.1).

C. Triturate 2 g with 2 ml of *water*; the resulting mixture flows.

Coarse particles. Transfer 5 g to a stoppered cylinder (16 cm × 35 mm), add 60 ml of a 1 per cent w/v solution of *sodium pyrophosphate*, shake thoroughly and allow to stand for 5 minutes. Using a pipette, withdraw 50 ml from a point about 5 cm below the surface of the liquid. To the remaining liquid add 50 ml of *water*, shake, allow to stand for 5 minutes and withdraw 50 ml in the same manner as before. Repeat the operation until a total of 400 ml of suspension has been withdrawn under the prescribed conditions. Transfer the remainder to an evaporating dish and evaporate to dryness on a water-bath. The residue, after drying at 105°, weighs not more than 25 mg.

Fine particles. Disperse 5 g in 250 ml of *water* by shaking vigorously for 2 minutes in a stoppered flask, pour immediately into a glass cylinder, 5 cm in diameter, and transfer 20 ml to a glass dish using a pipette. Evaporate to dryness and dry to constant weight at 105°. Allow the remainder of the suspension to stand for 4 hours at 20° and withdraw a second 20-ml portion using a pipette with its tip exactly 5 cm below the surface and without disturbing the sediment. Transfer the second portion to a glass dish, evaporate to dryness and dry to constant weight at 105°. The weight of the residue from the second portion is not less than 70 per cent of the weight of the residue from the first portion.

Tests

Arsenic (2.3.10). Disperse 5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*; the resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Heat 6.0 g for 15 minutes under a reflux condenser on a water-bath with a mixture of 70 ml of *water* and 10 ml of *hydrochloric acid* and filter. To 40 ml of the filtrate add 0.5 ml of *nitric acid* and evaporate to a low bulk. Add 20 ml of *water*, 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with two quantities, each of 10 ml, of a mixture of equal volumes of *amyl alcohol* and *ether*. To the aqueous layer add 2 g of *citric acid* and sufficient *water* to produce 60 ml. 12 ml of the solution complies with the limit test for heavy metals, Method D (20 ppm), using 10 ml of *lead standard solution (1 ppm pb)*.

Chlorides (2.3.12). Boil 1.5 g with 40 ml of *water* and 20 ml of *2 M nitric acid* under a reflux condenser for 5 minutes, cool and filter. 30 ml of the filtrate complies with the limit test for chlorides (330 ppm).

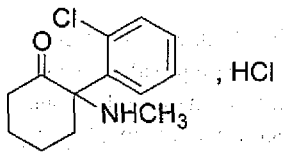
Soluble matter. Boil 2 g with 100 ml of *0.2 M hydrochloric acid* under a reflux condenser for 5 minutes, cool, filter and evaporate 50 ml to dryness. The residue, after ignition at about 600° for 30 minutes, weighs not more than 10 mg.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 1 g by drying in an oven at 105°.

Loss on ignition (2.4.20). Not more than 15.0 per cent, determined on 1 g by igniting at 600°.

Storage. Store protected from moisture.

Ketamine Hydrochloride



$C_{13}H_{16}ClNO,HCl$ Mol. Wt. 274.2

Ketamine Hydrochloride is (*RS*)-2-(2-chlorophenyl)-2-methylaminocyclohexanone hydrochloride.

Ketamine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{13}H_{16}ClNO,HCl$.

Category. General anaesthetic.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketamine hydrochloride IPRS* or with the reference spectrum of ketamine hydrochloride.

B. A 10 per cent w/v solution gives the reactions of chlorides (2.3.1).

C. Melting range (2.4.21). 258° to 261°.

Tests

Appearance of solution. A 20.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 3.5 to 4.1, determined in a 10.0 per cent w/v solution.

Optical rotation (2.4.22). – 0.2° to + 0.2°, determined in 2.0 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

– a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: dissolve 0.95 g of *sodium hexanesulphonate* in 1000 ml of a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water*, add 4 ml of *acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. Run the chromatogram 10 times the retention time of the principal peak. In the chromatogram obtained with the test solution the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh 0.5 g and dissolve in 1 ml of *anhydrous formic acid*. Add 50 ml of *anhydrous glacial acetic acid* and 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02742 g of $C_{13}H_{16}ClNO,HCl$.

Storage. Store protected from moisture.

Ketamine Injection

Ketamine Hydrochloride Injection

Ketamine Injection is a sterile solution of Ketamine Hydrochloride in Water for Injections.

Ketamine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ketamine, $C_{13}H_{16}ClNO$.

Usual strengths. The equivalent of 10 mg per ml; 50 mg per ml; 100 mg per ml (11.54 mg of ketamine hydrochloride is approximately equivalent to 10 mg of ketamine).

Identification

A. Dilute a suitable volume with *0.1 M hydrochloric acid* to produce a solution containing 0.03 per cent w/v of ketamine. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 269 nm and 276 nm.

B. Dilute a suitable volume with a mixture of 49 volumes of *methanol* and 1 volume of *1 M sodium hydroxide* to produce a solution containing 0.07 per cent w/v of ketamine. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 301 nm.

Tests

pH (2.4.24). 3.0 to 5.5.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dilute a quantity of injection containing about 0.12 g of Ketamine in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 2.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Lichrosorb RP18),
- mobile phase: a solution prepared by dissolving 0.95 g of *sodium hexanesulphonate* in 1000 ml of a mixture of 25 volumes of *acetonitrile* and 75 volumes of *water* and add 4 ml of *6 M acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 10 times the retention time of the principal peak. The retention time is about 3 to 4.5 minutes for ketamine. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), and the area of not more than one such peak is more than the area of the principal peak in the chromatogram obtained with reference solution (b) 0.25 per cent). Ignore any peak with an area less than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.40 Endotoxin Unit per mg of ketamine hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a volume of injection containing 0.5 g of ketamine add sufficient *water* to produce 200.0 ml and mix. To 20.0 ml of the resulting solution add 3 ml of *0.1 M sodium hydroxide* and extract with three quantities, each of 15 ml, of *chloroform*.

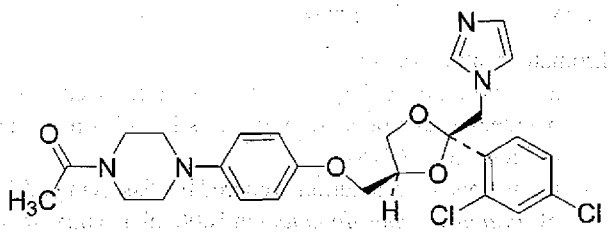
Combine the *chloroform* extracts and shake successively with three quantities, each of 30 ml, of *0.05 M sulphuric acid*. Dilute the combined acid extracts to 200.0 ml with *0.05 M sulphuric acid* (saturated with *chloroform*), and measure the absorbance of the resulting solution at the maximum at about 269 nm (2.4.7).

Calculate the content of $C_{13}H_{16}ClNO$ from the absorbance obtained from a solution of known concentration of *ketamine hydrochloride IPRS* in the same medium.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of ketamine in a suitable dose-volume.

Ketoconazole



$C_{26}H_{28}Cl_2N_4O_4$ Mol. Wt. 531.4

Ketoconazole is *cis*-1-acetyl-4-[[(2*RS*,4*RS*)-2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxyl]phenylpiperazine.

Ketoconazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{26}H_{28}Cl_2N_4O_4$, calculated on the dried basis.

Category. Antifungal.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketoconazole IPRS* or with the reference spectrum of ketoconazole.

B. Melting range (2.4.21). 148° to 152°.

Tests

Specific optical rotation (2.4.22). –1.0° to +1.0°, determined in a 4.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *methanol*.

Reference solution (a). Dissolve 2.5 mg, each of, *ketoconazole IPRS* and *loperamide hydrochloride IPRS* in 50 ml of *methanol*.

Reference solution (b). Dilute 5.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 1.0 ml of the solution to 10.0 ml with *methanol*.

Chromatographic system

- stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a mixture of 5 volumes of *acetonitrile* and 95 volumes of 0.34 per cent w/v solution of *tetrabutylammonium hydrogen sulphate*,
  B. a mixture of 50 volumes of *acetonitrile* and 50 volumes of 0.34 per cent w/v solution of *tetrabutylammonium hydrogen sulphate*,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
10	0	100
15	0	100
16	100	0
20	100	0

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to ketoconazole and loperamide is not less than 15.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.1 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 80° at a pressure not exceeding 2.7 kPa for 4 hours.

Assay. Weigh 0.2 g, dissolve in 40 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02657 g of $C_{26}H_{28}Cl_2N_4O_4$.

Storage. Store protected from light.

Ketoconazole Tablets

Ketoconazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ketoconazole, $C_{26}H_{28}Cl_2N_4O_4$.

Usual strength. 200 mg.

Identification

Determine by thin- layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 42 volumes of *hexane*, 40 volumes of *ethyl acetate,* 15 volumes of *methanol*, 2 volumes of *water* and 1 volume of *glacial acetic acid.*

Test solution. Disperse a quantity of powdered tablets containing 200 mg of Ketoconazole with 10 ml of *chloroform*, dilute to 20 ml with *chloroform* and filter.

Reference solution. A 1.0 per cent w/v solution of *ketoconazole IPRS* in *chloroform.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapour. The principal spot in the chromatogram obtained with the test solution corresponds to that obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary with the dissolution medium at 270 nm (2.4.7). Calculate the content of $C_{26}H_{28}Cl_2N_4O_4$, in the medium from the absorbances obtained from a solution of *ketoconazole IPRS.*

Q. Not less than 80 per cent of the stated amount of $C_{26}H_{28}Cl_2N_4O_4$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *dichloromethane.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 200 mg of Ketoconazole, shake with 50.0 ml of the solvent mixture and centrifuge. To 5.0 ml of the solution, add 5.0 ml of a 0.5 per cent w/v solution of *terconazole IPRS* (internal standard) in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

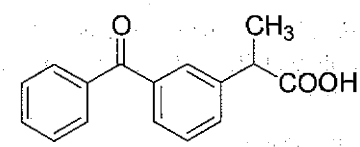
Reference solution. Dissolve 20 mg of *ketoconazole IPRS* in 20 ml of the solvent mixture, add 5.0 ml of the internal standard solution and dilute to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 7 volumes of 0.2 per cent w/v of *di-isopropylamine* in *methanol* and 3 volumes of a 0.5 per cent w/v solution of *ammonium acetate,*
- flow rate: 3 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. The relative retention times are about 0.6 for ketoconazole and 1.0 for terconazole.

Calculate the content of $C_{26}H_{28}Cl_2N_4O_4$ in the tablets.

Ketoprofen



$C_{16}H_{14}O_3$ Mol. Wt. 254.3

Ketoprofen is (*RS*)-2-(3-benzoylphenyl)propionic acid.

Ketoprofen contains not less than 98.5 per cent and not more than 100.5 per cent of $C_{16}H_{14}O_3$, calculated on the dried basis.

Category. Anti-inflammatory; analgesic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketoprofen IPRS* or with the reference spectrum of ketoprofen.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol (75 per cent)* shows an absorption maximum only at about 258 nm; absorbance at about 258 nm, about 0.66.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 50 volumes of *acetone*, 49 volumes of *dichloromethane* and 1 volume of *glacial acetic acid.*

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *acetone.*

Reference solution (a). A 0.1 per cent w/v solution of *ketoprofen IPRS* in *acetone*.

Reference solution (b). A mixture of equal volumes of 1.0 per cent w/v solution of *indomethacin IPRS* and refererence solution (a) in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 20 mg of the substance under examination in 20.0 ml of the mobile phase .

Reference solution. Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 2 volumes of freshly prepared *phosphate buffer pH 3.5*, 43 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 233 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. Run the chromatogram 7 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (0.2 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak due to impurity in the chromatogram obtained with the reference solution (0.4 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.02 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Weigh 0.5 g, dissolve in 25 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution*, add 25 ml of *water* Titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02543 g of $C_{16}H_{14}O_3$.

Storage. Store protected from moisture.

Ketoprofen Capsules

Ketoprofen Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ketoprofen, $C_{16}H_{14}O_3$.

Usual strengths. 50 mg; 100 mg.

Identification

A. Shake a quantity of the contents of the capsules containing 0.5 g of Ketoprofen with 50 ml of *chloroform* for 5 minutes, filter, evaporate to dryness using a rotary evaporator, induce crystallisation by prolonged scratching of the inside wall of the container with a glass rod and separate the crystals by centrifugation or filtration.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketoprofen IPRS* or with the reference spectrum of ketoprofen.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 258 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of phosphate buffer prepared by dissolving 1.46 g of *potassium dihydrogen orthophosphate* and 20.06 g of *disodium hydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.5 with *orthophosphoric acid*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary with the dissolution medium to prepare a solution containing about 0.001 per cent w/v of ketoprofen at 260 nm (2.4.7). Calculate the content of $C_{16}H_{14}O_3$, in the medium from the absorbances obtained from a solution of *ketoprofen IPRS* .

Q. Not less than 80 per cent of the stated amount of $C_{16}H_{14}O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*.

Test solution. Shake a quantity of the contents of capsules containing about 100 mg of Ketoprofen in 100 ml with the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 2 volumes of freshly prepared *phosphate buffer pH 3.5*, 43 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 233 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. The area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.02 per cent).

Other tests. Comply with the tests stated under Capsules.

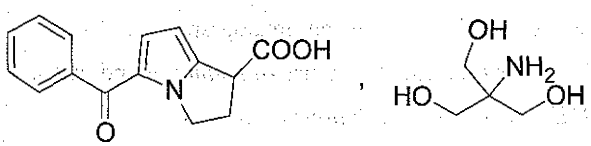
Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 25 mg of Ketoprofen, shake for 10 minutes with 150 ml of *methanol (75 per cent)*, mix and dilute to 250.0 ml with *methanol (75 per cent)*. Allow to stand, dilute 10.0 ml of the supernatant liquid to 100.0 ml with *methanol (75 per cent)* and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7).

Calculate the content of $C_{16}H_{14}O_3$ taking 662 as the specific absorbance at 258 nm.

Storage. Store protected from moisture.

Ketorolac Tromethamine

Ketorolac Trometamol



$C_{15}H_{13}NO_3,C_4H_{11}NO_3$ Mol. Wt. 376.4

Ketorolac Tromethamine is salt of (*RS*)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylate with 2-amino-2-(hydroxymethyl) propane-1,3-diol.

Ketorolac Tromethamine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$, calculated on the dried basis.

Category. Non-steroidal Anti-inflammatory.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketorolac tromethamine IPRS* or with the reference spectrum of ketorolac tromethamine.

B. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 95 volumes of *dichloromethane*, 5 volumes of *acetone* and 2 volumes of *glacial acetic acid*.

Test solution. A 0.5 per cent w/v solution of the substance under examination in a mixture of 2 volumes of *dichloromethane* and 1 volume of *methanol*.

Reference solution. A 0.5 per cent w/v solution of *ketorolac tromethamine IPRS* in the same solvent.

Apply to the plate 40 µl of each solution. After development, dry the plate in air, spray with a freshly prepared alcoholic solution containing 30 mg of *ninhydrin* per ml, dry at 150° for 2 to 5 minutes and examine. The yellow spots with pink to purple borders obtained with the test solution corresponds to the spots obtained with the reference solution.

Tests

Appearance of solution. A 3.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1.).

pH (2.4.24). 5.7 to 6.7 determined on a solution prepared by diluting 5 ml of solution A to 15 ml with *carbon dioxide-free water*.

Light absorption (2.4.7). Absorbance of solution A at 430 nm, not more than 0.10.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Solvent mixture. 30 volumes of *tetrahydrofuran* and 70 volumes of *water*.

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.04 per cent w/v solution of *ketorolac tromethamine IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml to 10.0 ml with the solvent mixture

Reference solution (c). Place in a 250-ml separating funnel, 100 ml of *water*, 100 ml of *dichloromethane*, 30 mg of *ketorolac tromethamine IPRS* and 1 ml of *1M hydrochloric acid*. Insert the stopper, shake, and allow the layers to separate. Transfer the lower dichloromethane layer to a stoppered borosilicate glass flask. Insert the stopper and expose to daylight for 10-15 minutes. Evaporate 1.0 ml of the solution to dryness in a current of air, or in a stream of nitrogen. Dissolve the residue in 1.0 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 30 volumes of *tetrahydrofuran* and 70 volumes of buffer solution prepared by dissolving 5.75 g of *ammonium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and diluting to 1000 ml with *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 313 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Unknown impurity[1]	0.54	2.2
Unknown impurity[2]	0.66	0.91
ketorolac 1-hydroxy analog	0.63	0.67
ketorolac 1-keto analog	0.89	0.52
ketorolac	1.0	—

[1]Unknown structure,

[2]Unknown structure.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to ketorolac 1-keto analog and ketorolac is not less than 1.5.

Inject reference solution (b) and the test solution. Run the chromatogram three times the retention time of the principal peak in the chromatogram obtained with the test solution the area of peak obtained with ketorolac 1-keto analog and ketorolac 1-hydroxy analog is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any other secondary peak is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 60° for 3 hours.

Assay. Determine by liquid chromatography as described under Related substances.

Inject reference solution (c) and (a). The test is not valid unless the resolution between ketorolac 1-keto analog and ketorolac is not less than 1.5 in the chromatogram obtained with reference solution (c), the column efficiency is not less than 5000 theoretical plates and the relative standard deviation for replicate injections is not more than 1.5 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{13}NO_3$,$C_4H_{11}NO_3$.

Ketorolac Tromethamine Injection

Ketorolac Trometamol Injection

Ketorolac Tromethamine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ketorolac tromethamine $C_{15}H_{13}NO_3$,$C_4H_{11}NO_3$.

Usual strengths. 15 mg per ml; 30 mg per ml.

Identification

Prepare a mixture of equal volumes of the test solution and reference solution (b) and chromatograph the mixture as directed in the Assay. The chromatogram thus obtained exhibits two main peaks corresponding to ketorolac and naproxen.

Tests

pH (2.4.24). 6.9 to 7.9.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 5.8 Endotoxin units per mg of ketorolac tromethamine.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water*.

NOTE — Protect the solutions from light.

Test solution. Dilute a measured volume containing 12 mg of Ketorolac Tromethamine to 50.0 ml with *methanol*. To 5.0 ml

of the solution add 5.0 ml of reference solution (b) and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.024 per cent w/v solution of *ketorolac tromethamine IPRS* in *methanol*. To 5.0 ml of the solution, add 5.0 ml of reference solution (b) and dilute to 50.0 ml with the solvent mixture.

Reference solution (b). A 0.03 per cent w/v solution of *naproxen IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *methanol*, 44 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

The relative retention times are about 0.7 for ketorolac and 1.0 for naproxen.

Inject reference solution (a). The resolution between the peaks due to ketorolac and naproxen is not less than 5.4, the column efficiency determined from the ketorolac peak is not less than 2700 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$ in the injection.

Storage. Store protected from light, at a temperature not exceeding 30°.

Ketorolac Tromethamine Tablets

Ketorolac Trometamol Tablets

Ketorolac Tromethamine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ketorolac tromethamine $C_{15}H_{13}NO_3,C_4H_{11}NO_3$.

Usual strength. 10 mg.

Identification

Prepare a mixture of equal volumes of the test solution and reference solution (a) and determine by liquid chromatography (2.4.14) as described under Assay, using the mixture. The chromatogram obtained exhibits two main peaks corresponding to ketorolac and naproxen.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 600 ml of *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance (2.4.7) of the filtrate, suitably diluted with the medium if necessary, at the maximum at about 322 nm.

Calculate the content of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$ in the medium from the absorbance obtained from a solution of known concentration of *ketorolac tromethamine IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$.

Uniformity of content. Complies with the test stated under Tablets.

Powder 1 tablet and transfer to volumetric flask that will provide a final concentration of about 0.1 mg of ketorolac tromethamine per ml. Add a quantity of *water* equivalent to about 10 per cent of the volume of the flask and mix with the aid of ultrasound. Add a quantity of *methanol* equivalent to about 40 per cent of the volume of the flask and mix with the aid of ultrasound for about 10 minutes to dissolve the ketorolac tromethamine. Cool, dilute to volume with *methanol*, mix and centrifuge. Dilute 5.0 ml of the clear supernatant to 50.0 ml with *methanol* and mix. Measure the absorbance of the resulting solution at the maximum at about 322 nm using *methanol* as blank. Calculate the content of $C_{15}H_{13}NO_3,C_4H_{11}NO_3$ from the absorbance obtained from a solution of known concentration of *ketorolac tromethamine IPRS*.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water*.

NOTE— Protect the solutions from light.

Test solution. Weigh and powder 20 tablets. Weigh a quantity of the powder containing about 20 mg of Ketorolac Tromethamine, add 10 ml of *water* and shake well. Add 40 ml of *methanol* and mix with the aid of ultrasound for about 10 minutes to dissolve the ketorolac tromethamine. Cool, dilute to 100.0 ml with *methanol* and mix. Centrifuge 5.0 ml of the clear supernatant liquid add 5.0 ml of reference solution (b), add sufficient solvent mixture to produce 50.0 ml and mix.

Reference solution (a). A 0.024 per cent w/v solution of *ketorolac tromethamine IPRS* in *methanol*. To 5.0 ml of the solution, add 5.0 ml of reference solution (b) and dilute to 50.0 ml with the solvent mixture.

Reference solution (b). A 0.03 per cent w/v solution of *naproxen IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase : a mixture of 55 volumes of *methanol*, 44 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

The relative retention times are about 0.7 for ketorolac and 1.0 for naproxen.

Inject reference solution (a). The resolution between the peaks due to ketorolac and naproxen is not less than 5.4, the column efficiency determined from the ketorolac peak is not less than 2700 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

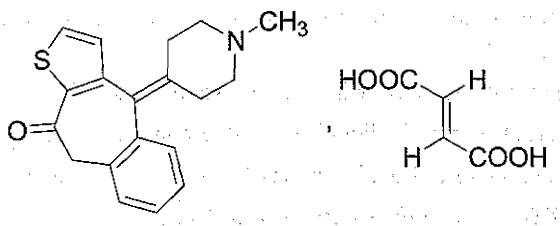
Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{13}NO_3$,$C_4H_{11}NO_3$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Ketotifen Fumarate

Ketotifen Hydrogen Fumarate



$C_{19}H_{19}NOS$,$C_4H_4O_4$ Mol. Wt. 425.5

Ketotifen Fumarate is 4,9-dihydro-4-(1-methylpiperidin-4-ylidene)-10*H*-benzo[4,5]cycloheptа[1,2-*b*]thiophen-10-one hydrogen fumarate.

Ketotifen Fumarate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{19}NOS$,$C_4H_4O_4$, calculated on the dried basis.

Category. Antihistaminic.

Description. A white to brownish-yellow crystalline powder.

Identification

A Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ketotifen hydrogen fumarate IPRS* or with the reference spectrum of ketotifen hydrogen fumarate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 3 volumes of *water*, 7 volumes of *anhydrous formic acid* and 90 volumes of *di-isopropyl ether*.

Test solution. Dissolve 40 mg of the substance under examination in 10.0 ml of *methanol*.

Reference solution. A 0.11 per cent w/v solution of *fumaric acid IPRS* in *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in warm air and examine under ultraviolet light at 254 nm. Spray lightly with a 0.5 per cent w/v solution of *potassium permanganate* in 1.4 per cent v/v solution of *sulphuric acid*. In the chromatogram obtained with the test solution, the spot due to fumaric acid corresponds to the principal spot in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 2.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution YS4, BYS4 or BS4 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Protect the solutions from light.

Solvent mixture. Equal volumes of *methanol* and *water*.

Test solution. Dissolve 30 mg of the substance under examination in 100.0 ml of a mixture of the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dissolve 3.0 mg of *4-(1-methylpiperidin-4-ylidene)-4H-benzo[4,5]cyclohepta[1,2-b]thiophen-9,10-dione IPRS* (*ketotifen impurity A IPRS*) in 10 ml of *methanol* and dilute to 20.0 ml with *water*.

Reference solution (c). To 1.5 ml of reference solution (b), add 1.0 ml of the test solution and dilute to 10 ml with the solvent mixture.

Reference solution (d). Dilute 0.5 ml of reference solution (b) to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 175 µl of *triethylamine* and 500 ml of *water*,
  B. a mixture of 175 µl of *triethylamine* and 500 ml of *methanol*,

- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 297 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	40	60
12	40	60
20	10	90
25	10	90
26	40	60
31	40	60

Inject reference solution (a), (c), (d) and the test solution. The relative retention time of ketotifen impurity A with reference to ketotifen is about 0.86. In the chromatogram obtained with reference solution (c), the resolution is not less than 1.5 between the peaks due to ketotifen and to ketotifen impurity A. In the chromatogram obtained with reference solution (d), the signal-to-noise ratio is not less than 70 for the principal peak.

For the calculation of contents, multiply the area of the corresponding peak by the following correction factor: 1.36 for impurity. In the chromatogram obtained with the test solution, the area of the peak due to ketotifen impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of the peak due to any other impurity is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 105° for 4 hours.

Assay. Dissolve 0.35 g in a mixture of 30 ml of *anhydrous acetic acid* and 30 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04255 g of $C_{23}H_{23}NO_5S$.

Storage. Store protected from light.

Ketotifen Fumarate Tablets

Ketotifen Fumarate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ketotifen, $C_{19}H_{19}NOS$.

Usual strength. 1 mg.

Identification

When examined in the range 200 nm to 400 nm (2.4.7). In the Assay, the solution of substance under examination shows absorption maximum at about 297 nm (2.4.7).

Tests

Uniformity of content. Complies with the test stated under Tablets, as described under Assay with the following modifications.

Test solution. Disperse 1 tablet in 50 ml *0.1 M hydrochloric acid* with the aid of ultrasound for 20 minutes and dilute to 100.0 ml with *0.1 M hydrochloric acid*, filter.

Calculate the content of $C_{19}H_{19}NOS$.

Other tests. Comply with the tests stated under Tablets.

Assay. *NOTE — Protect the solution from light.*

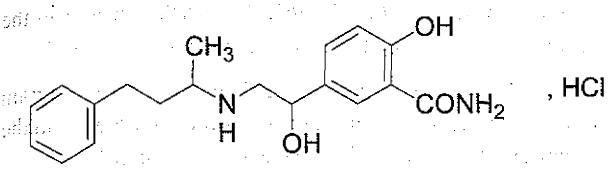
Weigh and powder 20 tablets. Disperse a quantity of powder containing 1 mg of Ketotifen in 70 ml of *0.1 M hydrochloric acid* with the aid of ultrasound for 20 minutes and dilute to 100.0 ml with *0.1 M hydrochloric acid*, filter and measure the absorbance of the resulting solution at the maximum at about 297 nm (2.4.7). Calculate the content of $C_{19}H_{19}NOS$ from the absorbance obtained by repeating the operation using *ketotifen fumarate IPRS* in place of the substance under examination.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of ketotifen.

L

Labetalol Hydrochloride



$C_{19}H_{24}N_2O_3$, HCl Mol. Wt. 364.9

Labetalol Hydrochloride is *all-rac*-2-hydroxy-5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl]benzamide hydrochloride.

Labetalol Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{24}N_2O_3$, HCl, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *labetalol hydrochloride IPRS* or with the reference spectrum of labetalol hydrochloride.

B. A 1 per cent w/v solution in *carbon dioxide-free water* (Solution A) gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 4.0 to 5.0, for solution A.

Diasterioisomer ratio. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 5 mg of the substance under examination in 1.0 ml of 1.2 per cent w/v solution of *1-butane boronic acid* in *anhydrous pyridine* and allow to stand for 20 minutes.

Chromatographic system
- a fused silica column 25 m x 0.53 mm, coated with methyl silicone polymer (film thickness 5.0 µm) (Such as DB-17),
- temperature:
  column. 290°,
  inlet port and detector at 350°,
- flame ionization detector,
- flow rate: 20 ml per minute, using nitrogen as the carrier gas.

Inject 2 µl of the test solution. Two peaks due to the two diasterioisomers appear in the chromatogram. The test is not valid unless the resolution between the two labetalol peaks is not less than 1.5 and the relative standard deviation of replicate injections for the ratio of the area of first peak to the secondary peak is not more than 2.0 per cent.

The ratio of the area of secondary peak to the sum of the areas of the two adjacent peaks is not less than 45 per cent and not more than 55 per cent.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A. Further dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution (b). Dissolve 5 mg of *labetalol impurity A IPRS* in 50 ml of mobile phase B, add 1.0 ml of the test solution and dilute to 100.0 ml with mobile phase A.

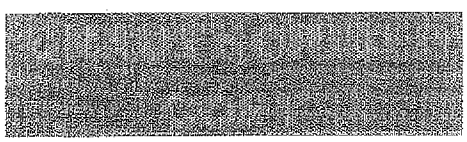
Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with end-capped ethylene-bridged octadecylsilane bonded to porous silica (3.5 µm),
- column temperature: 40°,
- mobile phase: A. 0.1 per cent v/v solution of *ortho-phosphoric acid* in *water*,
  B. a mixture of equal volumes of *acetonitrile* and mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
40	0	100
45	0	100
45.1	100	0
50	100	0

The relative retention time with reference to labetalol (retention time: about 22 minutes) for labetalol impurity A is about 1.1.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to labetalol and labetalol impurity A is not less than 4.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference



solution (a) (0.05 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Test solution. Dissolve 50 mg of the substance under examination in mobile phase A and dilute to 100.0 ml with the same solvent. Dilute to 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution. A 0.005 per cent w/v solution of *labetalol hydrochloride IPRS* in mobile phase A.

- mobile phase: a mixture of 45 volumes of mobile phase A and 55 volumes of mobile phase B.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{24}N_2O_3$, HCl.

Storage. Store protected from moisture, at temperature not exceeding 30°.

Labetalol Injection

Labetalol Hydrochloride injection

Labetalol Injection is a sterile solution of Labetalol Hydrochloride in Water for Injections.

Labetalol Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of labetalol hydrochloride, $C_{19}H_{24}N_2O_3$,HCl.

Usual strength. 5 mg per ml.

Description. A colorless or pale yellow solution.

Identification

A. Mix a volume containing 50 mg of Labetalol Hydrochloride with 50 ml of *0.1 M hydrochloric acid* and heat on a water-bath for 30 minutes. Cool, filter, add 10 ml of *ammonia buffer pH 10.0* and extract with three 15-ml quantities of *dichloromethane*. Shake the combined extracts with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *labetalol hydrochloride IPRS*, treated in the same manner or with the reference spectrum of labetalol.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 3.5 to 4.5.

Free carboxylic acid and other related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 5 volumes of *13.5 M ammonia*, 25 volumes of *methanol* and 75 volumes of *dichloromethane*.

Test solution. Dilute a volume of the injection containing 80 mg of Labetalol Hydrochloride to 50.0 ml with *ethanol (95 per cent)*, evaporate to dryness using a rotary evaporator and dissolve the residue in 1 ml of *methanol*.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with *methanol*.

Reference solution (b). A 0.16 per cent w/v solution of *5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl] salicylic acid hydrochloride IPRS* in *methanol*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air, heat at 105° for 30 minutes, cool and examine under ultraviolet light at 254 nm. The area of any spot corresponding to 5-[1-hydroxy-2-(1-methyl-3- phenylpropylamino)ethyl]salicylic acid is not more intense that the spot in the chromatogram obtained with reference solution (b) (2.0 per cent), the area of any other secondary peak is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Bacterial endotoxins (2.2.3). Not more than 1.2 Endotoxin Units per mg of labetalol hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing 50 mg of Labetalol Hydrochloride to 100.0 ml with the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *labetalol hydrochloride IPRS* in the mobile phase.

Reference solution (b). A 0.008 per cent w/v solution of *methylparaben IPRS* in reference solution (a).

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 1),
- column temperature: 60°,
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 13.8 g of *monobasic sodium phosphate* in 1000 ml of *water* and 35 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 5 µl.

The relative retention time with reference to labetalol for methylparaben is about 0.6.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to methylparaben and labetalol is not less than 2.0 in the chromatogram obtained with reference solution (b), the column efficiency in not less than 700 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{19}H_{24}N_2O_3$, HCl in the injection.

Storage. Store protected from light, at temperature not exceeding 30°. Do not freeze.

Labetalol Tablets

Labetalol HydrochlorideTablets

Labetalol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of labetalol hydrochloride, $C_{19}H_{24}N_2O_3$,HCl.

Usual strengths. 50 mg; 100 mg; 200 mg; 400 mg.

Identification

A. To a quantity of the powdered tablets containing 50 mg of Labetalol Hydrochloride add 50 ml of *0.1 M hydrochloricacid* and heat on a water-bath for 30 minutes. Cool, filter, add 10 ml of *ammonia buffer pH 10.0* and extract with three quantities, each of 15 ml, of *dichloromethane*. Shake the combined extracts with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *labetalol hydrochloride IPRS* treated in the same manner or with the reference spectrum of labetalol.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, dilute suitably if necessary with the medium, at the maximum at about 302 nm (2.4.7). Calculate the content of $C_{19}H_{24}N_2O_3$, HCl in the medium from the absorbance obtained from a solution of known concentration of *labetalol hydrochloride IPRS* in dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{19}H_{24}N_2O_3$, HCl.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *dichloromethane*, 25 volumes of *methanol* and 5 volumes of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 0.5 g of Labetalol Hydrochloride with 10 ml of *methanol*, filter and use the filtrate.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*.

Reference solution (b). Dilute reference solution (a) with an equal volume of *methanol*.

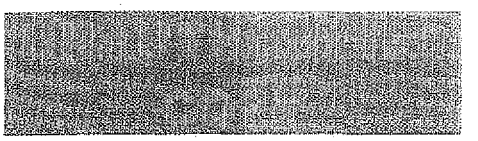
Apply to the plate 20 µl of each solution. After development, dry the plate in a current of warm air, heat at 105° for 30 minutes, cool and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (1.0 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.4 g of Labetalol Hydrochloride in 40 ml of *water*, shake by mechanical means for 60 minutes and dilute to 100.0 ml with *water*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.04 per cent w/v solution of *labetalol hydrochloride IPRS* in the mobile phase.



Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS1),
- column temperature: 60°,
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 13.8 g of *sodium dihydrogen phosphate* in 1000 ml of *water* and 35 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 5 µl.

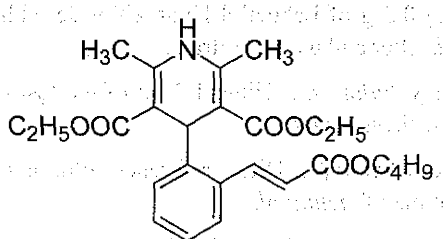
Inject the reference solution. The test is not valid unless the column efficiency is not less than 700 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{24}N_2O_3$,HCl in the tablets.

Storage. Store protected from moisture, at temperature not exceeding 30°.

Lacidipine



$C_{26}H_{33}NO_6$ Mol. Wt. 455.6

Category. Antihypertensive.

Lacidipine is diethyl (*E*)-4-2-[(*tert*-butoxycarbonyl) vinyl]phenyl-1,4-dihydro-2,6- dimethylpyridine-3,5-dicarboxylate.

Lacidipine contains not less than 97.5 per cent and not more than 102.0 per cent of $C_{26}H_{33}NO_6$, calculated on the anhydrous and propan-2-ol free basis.

Description. A white to pale yellow crystalline powder.

NOTE — Carry out all of the following procedures protected from light and prepare solutions immediately before use.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lacidipine IPRS* or with the reference spectrum of lacidipine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Melting point (2.4.21). It melts at about 178°.

Propan-2-ol. Not more than 0.5 per cent w/w.

Determine by gas chromatography (2.4.13).

Solution A. A 0.002 per cent v/v solution of *1,4-dioxan* (internal standard) in *dimethylacetamide*.

Test solution. A 2.0 per cent w/v solution of the substance under examination in solution A.

Reference solution. A 0.002 per cent w/v solution of *propan-2-ol* in solution A.

Chromatographic system
- a capillary column 60 m x 0.32 mm, bonded with a film of *polymethylsiloxane* (5 µm) (Such as CP-Sil 5CB),
- temperature:

column	time (min.)	temperature (°)
	0-1	60
	1-18	60-110
	18-20	110-200
	20-27	200

- inlet port. 170° and detector.250°,
- flow rate: 1.7 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the chromatogram shows two clearly separated peaks.

The retention time for propan-2-ol is about 6.2 minutes and that for dioxan is about 15 minutes.

Inject 1 µl of the reference solution and the test solution.

Calculate the content of propan-2-ol.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute 1.0 ml of a 0.1 per cent w/v solution of the substance under examination in *ethanol* to 5.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 500.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of 0.1 per cent w/v solution of *lacidipine impurity standard IPRS* in *ethanol* to 5.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with cyanosilane bonded to porous silica (5 µm) (Such as Spherisorb CN),

- mobile phase: a mixture of 3 volumes of *ethanol* and 97 volumes of n-*hexane*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the chromatogram obtained resembles the chromatogram supplied with *lacidipine impurity standard IPRS*.

The retention time of lacidipine peak is about 10 minutes.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to lacidipine impurity B ((diethyl (*E*)-4-2-[2-(*tert*-butoxycarbonyl)vinyl]phenyl-2,6-dimethylpyridine-3,5-dicarboxylate) is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent taking into account the correction factor of 0.5), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with a relative retention time of 1.5 with respect to the peak due to lacidipine impurity B.

Water (2.3.43). Not more than 0.2 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute 5 volumes of a 0.1 per cent w/v solution of the substance under examination in *ethanol* to 100 volumes with the mobile phase.

Reference solution. Dilute 5 volumes of a 0.1 per cent w/v solution of *lacidipine IPRS* in *ethanol* to 100 volumes with the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{33}NO_6$.

Lacidipine Tablets

Lacidipine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lacidipine, $C_{26}H_{33}NO_6$.

Usual strengths. 2 mg; 4 mg

NOTE — Carry out all of the following procedures protected from light and prepare solutions immediately before use.

Identification

A. Disperse a quantity of whole tablets containing 4 mg of Lacidipine with 50 ml of *ethanol* with the aid of ultrasound for 30 minutes. Dilute to 100 ml with *ethanol* and filter through a 0.45-µm membrane filter. When examined in the range 250 nm to 400 nm (2.4.7), exhibits maxima only at 284 nm and 368 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 500 ml of a solution prepared by mixing 100 ml of *water* with 10 ml of *polysorbate 20* and diluting to 1000 ml with *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a 0.45-µm membrane filter, having first activated the filter with 3 ml of *methanol* followed by 5 ml of a 1.0 per cent w/v solution of *polysorbate 20*. Measure the absorbance of the resulting solution at the maximum at about 284 nm (2.4.7). Calculate the content of lacidipine, $C_{26}H_{33}NO_6$ in the medium from the absorbance obtained from a solution containing 0.02 per cent w/v of *lacidipine IPRS* prepared by dissolving in minimum amount of ethanol and diluting with dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{26}H_{33}NO_6$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 20 mg of Lacidipine with 50 ml of *ethanol* with the aid of ultrasound for 15 minutes. Cool, dilute to 100 ml with *ethanol*, filter through a 0.45-µm membrane filter. Dilute 5.0 ml of the solution to 20.0 ml with *hexane*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of 0.1 per cent w/v solution of *lacidipine impurity standard IPRS* in *ethanol* to 5.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with cyanosilane bonded to porous silica (5 µm) (Such as Spherisorb CN),
- mobile phase: a mixture of 3 volumes of *ethanol* and 97 volumes of n-*hexane*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the chromatogram obtained resembles the chromatogram supplied with *lacidipine impurity standard IPRS*.

The retention time of lacidipine peak is about 10 minutes.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to lacidipine impurity B ((diethyl (*E*)-4-2-[2-(*tert*-butoxycarbonyl)vinyl]phenyl-2,6-dimethylpyridine-3,5-dicarboxylate) is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent taking into account the correction factor of 0.5), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent). Ignore any peak with a relative retention time of 1.5 with respect to the peak due to lacidipine impurity B.

Uniformity of content. Complies with the test stated under Tablets.

Disperse 1 tablet in 50 ml of *ethanol* with the aid of ultrasound for 30 minutes. Cool and filter through a 0.45-µm membrane filter and dilute with *ethanol* to produce a solution containing 0.004 per cent w/v of Lacidipine. Measure the absorbance at the maximum at 368 nm (2.4.7).

Calculate the content of $C_{26}H_{33}NO_6$ in the tablet from the absorbance obtained from a 0.004 per cent w/v solution of *lacidipine IPRS* in *ethanol*.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of lacidipine in 35 ml *ethanol* with the aid of ultrasound for 30 minutes. Cool and filter through a 0.45 µm membrane filter and dilute to 200.0 ml with mobile phase.

Reference solution (a). Dilute 5.0 ml of a 0.04 per cent w/v solution of *lacidipine IPRS* in *ethanol* to 20.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of 0.1 per cent w/v solution of *lacidipine impurity standard IPRS* in *ethanol* to 5.0 ml with the mobile phase.

Inject reference solution (b). The test is not valid unless the chromatogram obtained resembles the chromatogram supplied with *lacidipine impurity standard IPRS*.

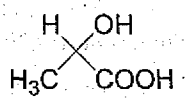
Use chromatographic system as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{26}H_{33}NO_6$ in the tablets.

Storage. Store protected from light.

Lactic Acid



$C_3H_6O_3$ Mol. Wt. 90.1

Lactic Acid consists of a mixture of 2-hydroxypropionic acid, its condensation products, such as lactoyl-lactic acid and other polylactic acids and water. The equilibrium between lactic acid and polylactic acids depends on concentration and temperature.

It is usually in the form of the racemate [(*RS*)-lactic acid], but in some cases the (*S*)-isomer may predominate.

Lactic Acid contains the equivalent of not less than 88.0 per cent and not more than 92.0 per cent w/w of $C_3H_6O_3$.

Category. Pharmaceutical aid.

Description. A colourless or slightly yellow, viscous liquid; hygroscopic.

Identification

A. Warm 1 g with 0.1 g of *potassium permanganate*; acetaldehyde is evolved.

B. It gives reaction (A) of lactates (2.3.1).

C. A 10 per cent w/v solution is strongly acidic (2.4.46).

Tests

Appearance of solution. The substance under examination is not more intensely coloured than reference solution YS6 (2.4.1).

Arsenic (2.3.10). Mix 10.0 g with 50 ml of *water* and 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Citric, oxalic and phosphoric acids. To 5 ml of the solution prepared in the test for Heavy metals add *6 M ammonia* until slightly alkaline. Add 1 ml of *calcium chloride solution* and heat on a water-bath for 5 minutes. Both before and after heating, any opalescence in the solution is not more intense than that in a mixture of 5 ml of the test solution and 1 ml of *water*.

Ether-insoluble substances. Dissolve 1.0 g in 25 ml of *ether*; the solution is not more opalescent than the solvent used for the test.

Volatile fatty acids. Cautiously heat 5 g in a glass-stoppered flask at 50° for 10 minutes; no unpleasant odour resembling that of the lower fatty acids is recognisable immediately after opening the flask.

Methanol and methyl esters. Place 2.0 g in a ground-glass-stoppered round-bottom flask and add 10 ml of *water*. Cool in ice, cautiously add 30 ml of a 30 per cent w/v solution of *potassium hydroxide* and cool in ice for a further 10 to 15 minutes. Steam distil the mixture into a 10-ml graduated cylinder containing 1 ml of *ethanol*, collecting a volume of at least 9.5 ml and dilute to 10.0 ml with *water*. To 1.0 ml of the distillate add 5 ml of *potassium permanganate and phosphoric acid solution* and mix. After 15 minutes add 2 ml of *oxalic acid and sulphuric acid solution*, stir with a glass rod until the solution is colourless and then add 5 ml of *decolorised magenta solution*. After 2 hours any colour in the solution is not more intense than that of 1 ml of a reference solution containing 100 µg of *methanol* and 0.1 ml of *ethanol* treated in the same manner beginning at the words "add 5 ml of *potassium permanganate* and *phosphoric acid solution*..".

Reducing sugars. Dilute 1 g with 10 ml of *water*, neutralise with *sodium hydroxide solution*, add 5 ml of *potassium cupri-tartrate solution* and boil; no red or greenish precipitate is produced.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

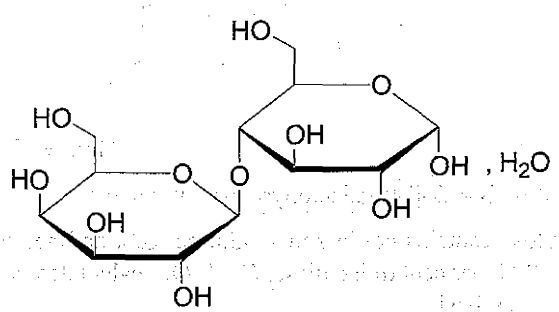
Assay. Weigh 1.0 g in a ground-glass-stoppered flask and add 10 ml of *water*. Add 20.0 ml of *1 M sodium hydroxide*, stopper the flask and allow to stand for 30 minutes. Titrate the excess of alkali with *1 M hydro-chloric acid*, using *dilute phenolphthalein solution* as indicator until the pink colour is discharged.

1 ml of *1 M sodium hydroxide* is equivalent to 0.09008 g of $C_3H_6O_3$.

Storage. Store protected from light.

Lactose

Lactose Monohydrate; Milk Sugar



$C_{12}H_{22}O_{11},H_2O$ Mol. Wt. 360.3

Lactose is *O*-β-D-galactopyranosyl-(1 → 4)-α-D-glucopyranose monohydrate.

Category. Pharmaceutical aid (excipient).

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lactose IPRS* or with the reference spectrum of lactose.

B. To 5 ml of a saturated solution add 5 ml of *1 M sodium hydroxide* and gently warm the mixture; the liquid becomes yellow and then brownish-red. Cool to room temperature and add 0.2 ml of *potassium cupri-tartrate solution*; a red precipitate is formed.

C. Heat 5 ml of a 5 per cent w/v solution with 5 ml of *10 M ammonia* in a water-bath at 80° for 10 minutes; a red colour develops.

Tests

Appearance of solution. Dissolve 1.0 g in *water* by heating to 50°, dilute to 10 ml with *water* and allow to cool. The solution is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

Acidity or alkalinity. Dissolve 6 g in 25 ml of *carbon dioxide-free water* by boiling, cool and add 0.3 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.4 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Specific optical rotation (2.4.22). + 54.4° to + 55.9°, determined in a solution obtained by dissolving 10.0 g in 80 ml of *water* by heating to 50°, allowing to cool, adding 0.2 ml of *6 M ammonia*, allowing to stand for 30 minutes and diluting to 100.0 ml with *water*.

Light absorption (2.4.7). Dissolve 1.0 g in boiling *water* and dilute to 10 ml with the same solvent (solution A). Absorbance of solution A measured at the maximum at about 400 nm, not more than 0.04.

Dilute 1 ml of solution A to 10 ml with *water*.

When examined in the range 210 nm to 300 nm, absorbance is not more than 0.25 in the range 210 nm to 220 nm and not more than 0.07 in the range 270 nm to 300 nm.

Arsenic (2.3.10). Dissolve 10.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*; the resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). Dissolve 4.0 g in 20 ml of warm *water*, 1.0 ml of *0.1 M hydrochloric acid* and sufficient *water* to

produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

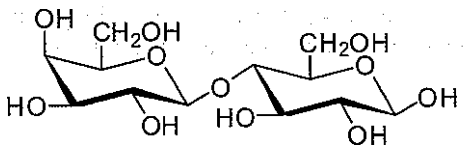
Microbial contamination (2.2.9). Total microbial count is not more than 10^2 CFU per g. 1 g is free from *Escherichia coli* and 10 g is free from *Salmonella* and *Shigella*.

Sulphated ash. Not more than 0.1 per cent, determined in the following manner. To 1.0 g add 1 ml of *sulphuric acid*, evaporate to dryness on a water-bath and ignite to constant weight.

Water (2.3.43). 4.5 to 5.5 per cent, determined on 0.5 g in a mixture of 1 volume of *formamide* and 2 volumes of *methanol*.

Storage. Store protected from moisture.

Anhydrous Lactose



$C_{12}H_{22}O_{11}$ Mol. Wt. 342.3

Anhydrous Lactose is 4-*O*-β-D-galactopyranosyl-D-glucose.

Category. Pharmaceutical aid (excipient).

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *anhydrous lactose IPRS* or with the reference spectrum of anhydrous lactose.

B. To 5 ml of a saturated solution add 5 ml of *1 M sodium hydroxide* and gently warm the mixture; the liquid becomes yellow and then brownish-red. Cool to room temperature and add 0.2 ml of *potassium cupri-tartrate solution*; a red precipitate is formed.

C. Heat 5 ml of a 5.0 per cent w/v solution with 5 ml of *10 M ammonia* in a water-bath at 80° for 10 minutes; a red colour develops.

Tests

Appearance of solution. Dissolve 1.0 g in *water* by heating to 50°, dilute to 10 ml with *water* and allow to cool. The solution is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

Acidity or alkalinity. Dissolve 6 g of the substance under examination in 25 ml of *carbon dioxide-free water* by boiling, cool and add 0.3 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.4 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Specific optical rotation (2.4.22). +54.4° to +55.9°, determined in a solution prepared by dissolving 10 g in 80 ml of *water* by heating to 50°, allow to cool and add 0.2 ml of *6 M ammonia*. Allow to stand for 30 minutes and dilute to 100.0 ml with *water*.

Light absorption (2.4.7). A 10.0 per cent w/v solution in *water*, shows an absorption maxima at about 400 nm is not more than 0.04. Dilute 1.0 ml of the solution to 10.0 ml with *water*. When examined in the range 210 nm to 220 nm; absorbance is not more than 0.25 and in the range 270 nm to 300 nm, absorbance is not more than 0.07.

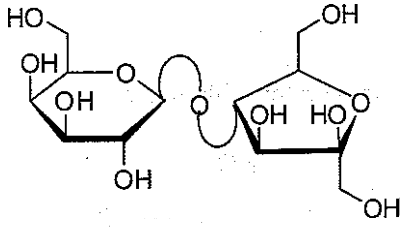
Heavy metals (2.3.13). Dissolve 4 g in 20.0 ml of *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D (5 ppm) using 10 ml of *lead standard solution (1 ppm, Pb)*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g in a mixture of 1 volume of *formamide* and 2 volumes of *methanol*.

Microbial contamination (2.2.9). Total microbial count not more than 100 CFU per g, 1 g is free from *Escherichia coli*.

Lactulose



$C_{12}H_{22}O_{11}$ Mol. Wt. 342.3

Lactulose is 4-*O*-β-D-galactopyranosyl-D-fructose.

Lactulose contains not less than 95.0 per cent and not more than 102.0 per cent of lactulose, $C_{12}H_{22}O_{11}$, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out and test B may be omitted if tests A, C and D are carried out.

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 10 volumes of *glacial acetic acid*, 15 volumes of 5 per cent w/v solution of *boric acid*, 20 volumes of *methanol* and 55 volumes of *ethyl acetate*.

Test solution. Dissolve 50 mg of the substance under examination in 10.0 ml of *water*.

Reference solution. A 0.5 per cent w/v solution of *lactulose IPRS* in *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 105° for 5 minutes and allow to cool. Spray with a 0.1 per cent w/v solution of *1,3-dihydroxynaphthalene* in a mixture of 10 volumes of *sulphuric acid* and 90 volumes of *methanol*. Heat at 110° for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

C. Dissolve 50 mg in 10 ml of *water*. Add 3 ml of *cupri-tartaric solution* and heat. A red precipitate is formed.

D. Dissolve 0.125 g in 5 ml of *water* and add 5 ml of *ammonia*. Heat on a water-bath at 80° for 10 minutes. A red colour develops.

Tests

Solution A. Dissolve 3.0 g in 50.0 ml of *carbon dioxide-free water*.

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution BYS5 (2.4.1).

pH (2.4.24). 3.0 to 7.0, determined in solution A.

Specific optical rotation (2.4.22). −50.0° to −46.0°, determined in a 5 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 1 g of the substance under examination in 10 ml of *water*. Add 12.5 ml of *acetonitrile* with gentle heating and dilute to 25.0 ml with *water*.

Reference solution (a). To 3 ml of the test solution, add 47.5 ml of *acetonitrile* with gentle heating and dilute to 100.0 ml with *water*.

Reference solution (b). Dissolve 1.0 g of *lactulose IPRS* in 10 ml of *water*, add 12.5 ml of *acetonitrile* with gentle heating and dilute to 25.0 ml with *water*.

Reference solution (c). Dissolve the contents of a vial of *lactulose for system suitability IPRS* in 1 ml of a mixture of equal volumes of *acetonitrile* and *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with aminopropylsilane bonded to porous silica (3 µm),
- column temperature: 38°,
- mobile phase: a mixture of 220 volumes of a solution prepared by dissolving 1.15 g of *sodium dihydrogen orthophosphate* in 1000 ml of *water* and 780 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- refractive index detector,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to lactulose and lactulose impurity A is not less than 1.3. The relative retention time with reference to lactulose for tagatose (lactulose impurity E) is about 0.38, for fructose (lactulose impurity D) is about 0.42, for galactose (lactulose impurity B) is about 0.57, for epilactose (lactulose impurity A) is about 0.9 and for lactose (lactulose impurity C) is about 1.17.

Inject reference solution (a) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

Methanol. Not more than 50 ppm.

Determine by gas chromatography (2.4.13).

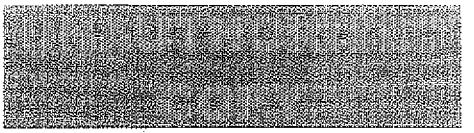
Internal standard solution. Mix 0.5 ml of *propanol* with 100.0 ml of *water*. Dilute 1.0 ml of the solution to 100.0 ml with *water*. Dilute 5.0 ml of the solution to 50.0 ml with *water*.

Test solution. To 79 mg of the substance under examination in a 20 ml vial, add 1.0 ml of the internal standard solution and 5 µl of a 0.1 per cent v/v solution of *methanol*.

Reference solution. To 1.0 ml of the internal standard solution in a 20 ml vial, add 5 µl of a 0.1 per cent v/v solution of *methanol*.

Chromatographic system
- a stainless steel column 2 m x 2 mm, packed with ethylvinylbenzene-divinylbenzene copolymer (180 µm),
- column temperature: 140°,
- inlet port at 200° and detector at 220°,
- flame ionization detector,
- flow rate: 30 ml per minute, helium as the carrier gas.

Head space conditions
- equilibration temperature: 60°,



– equilibration time 1 hour,
– pressurisation time: 1 minute.

Inject 1ml of the reference solution and the test solution.

Calculate the content of methanol, taking its density at 20° to be 0.79 g per ml.

The ratio (*R*) of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the reference solution and the ratio of the area of the peak due to methanol to the area of the peak due to the internal standard in the chromatogram obtained with the test solution is not more than 2*R* (50 ppm).

Boron. Not more than 9 ppm.

NOTE—Avoid where possible the use of glassware.

Reference solution. Dissolve 50 mg of *boric acid* in 100.0 ml of *water*. Dilute 5.0 ml of the solution to 100.0 ml with *water*. *Keep in a well-closed polyethylene container.*

In first polyethylene 25-ml flask, dissolve 0.5 g of the substance under examination in 2.0 ml of *water* (solution A), in second polyethylene 25-ml flask, dissolve 0.5 g of the substance under examination dissolved in 1.0 ml of the reference solution and 1.0 ml of *water* (solution B), in third polyethylene 25 ml flask, dilute 1.0 ml of the reference solution with 1.0 ml of *water* (solution C) and in fourth polyethylene 25 ml flask, take 2.0 ml of *water* (solution D).

To each flask, add 4.0 ml of *acetate-edetate buffer solution pH 5.5*. Mix and add 4.0 ml of freshly prepared *azomethine H solution*. Mix and allow to stand for 1 hour. Measure the absorbance of solutions A, B and C at 420 nm (2.4.7), using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is not less than 0.25. The absorbance of solution B is not less than twice that of solution A.

Lead. Not more than 0.5 ppm.

Determine by atomic absorption spectrometry (2.4.2).

Solvent mixture. Equal volumes of *dilute acetic acid* and *water*.

Test solution. Dissolve 20.0 g of the substance under examination in 100 ml of the solvent mixture. Add 2.0 ml of 1 per cent w/v solution of *ammonium pyrrolidinedithiocarbamate* and 10.0 ml of *methyl isobutyl ketone*, shake for few seconds protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solution (a). Dilute 0.5 ml of *lead standard solution (10 ppm Pb)* to 100 ml with the solvent mixture. Add 2.0 ml of a clear 1 per cent w/v solution of *ammonium pyrrolidinedithiocarbamate* and 10.0 ml of *methyl isobutyl ketone*, shake for few seconds and protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solution (b). Dilute 1.0 ml of *lead standard solution (10 ppm Pb)* to 100 ml with the solvent mixture. Add 2.0 ml of a clear 1 per cent w/v solution of *ammonium pyrrolidinedithiocarbamate* and 10.0 ml of *methyl isobutyl ketone*, shake for few seconds and protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solution (c). Dilute 1.5 ml of *lead standard solution (10 ppm Pb)* to 100 ml with the solvent mixture. Add 2.0 ml of a clear 1 per cent w/v solution of *ammonium pyrrolidinedithiocarbamate* and 10.0 ml of *methyl isobutyl ketone*, shake for few seconds and protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Set the zero of the instrument using *methyl isobutyl ketone* treated as described for the test solution without the substance under examination. Measure the absorbance at 283.3 nm using a lead hollow-cathode lamp as source of radiation and an air-acetylene flame.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.5 per cent, determined on 0.5 g.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 10^2 CFU per g determined by plate count. 1 g is free from *Escherichia coli*.

Assay. Determine by liquid chromatography (2.4.14), as described under test for Related substances with the following modification.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{12}H_{22}O_{11}$.

Lactulose Oral Powder

Lactulose Oral Powder consists of lactulose with or without lesser amounts of other sugars including lactose and galactose.

Lactulose Oral Powder contains not less than 95.0 per cent and not more than 102.0 per cent of the stated amount of lactulose, $C_{12}H_{22}O_{11}$.

Identification

A. Determine by thin-layer chromatography, (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 10 volumes of *glacial acetic acid*, 15 volumes of a 5 per cent w/v solution of *boric acid*, 20 volumes of *methanol* and 55 volumes of *ethyl acetate*.

Test solution. Dissolve 50 mg of the oral powder in *water* and dilute to 10.0 ml with *water*.

Reference solution. A 0.5 per cent w/v solution of *lactulose IPRS* in *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate at 100° to 105° for 5 minutes. Spray the plate with a 0.1 per cent w/v solution of *1,3-dihydroxynaphthalene* in a mixture of 10 volumes of *sulphuric acid* and 90 volumes of *methanol*. Heat the plate at 110° for 5 minutes. The principal spot in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. Dissolve 0.05 g in 10 ml of *water*, add 3 ml of *cupri-tartaric solution* and heat; a red precipitate is formed.

D. Dissolve 0.125 g in 5 ml of *water*, add 5 ml of *ammonia*. Heat on a water-bath at 80° for 10 minutes; a red colour develops.

E. Specific optical rotation. (See tests).

Tests

Solution A. A 6.0 per cent w/v solution of the substance under examination in *carbon dioxide-free water*.

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution BYS5 (2.4.1).

pH (2.4.24). 3.0 to 7.0, determined on a solution prepared by adding 0.1 ml of a saturated solution of *potassium chloride* to 10 ml of solution A.

Specific optical rotation (2.4.22). –50.0° to –46.0°, determined in a solution prepared by dissolving 1.25 g in *water*, adding 0.2 ml of *13.5 M ammonia* and diluting to 25.0 ml with *water*.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the sum of the areas of peaks corresponding to galactose, lactose, epilactose, tagatose and fructose is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent).

Methanol. Not more than 50 ppm.

Determine by Head-space gas chromatography (2.4.13).

NOTE — Maintain each solution at 60° for 1 hour, pressurize for 1 minute and transfer on the column 1 ml of the gaseous phase.

Internal standard solution. Dilute 0.5 ml of *propan-1-ol* to 100.0 ml with *water*. Dilute 1.0 ml of the solution to 100.0 ml with *water*. Further dilute 5.0 ml of the solution to 50.0 ml with *water*.

Test solution. To 79 mg of the oral powder, add 1 ml of the internal standard solution and 5 µl of a 0.1 per cent v/v solution of *methanol*.

Reference solution. Add 5 µl of a 0.1 per cent v/v solution of *methanol* to 1 ml of the internal standard solution.

Chromatographic system

- a column 2 m x 2 mm packed with ethylvinylbenzene–divinylbenzene co-polymer (film thickness 180 µm),
- temperature:
  column: 140°,
  inlet port at 200° and detector at 220°,
- a flame ionisation detector,
- flow rate: 30 ml per minute, using nitrogen as carrier gas.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the ratio of the area of the methanol peak to that of the internal standard peak is not more than twice the corresponding ratio for the chromatogram obtained with the reference solution.

Calculate the content of methanol assuming the density of methanol to be 0.79 g per ml at 20°.

Boron. *NOTE — Avoid where possible the use of glassware.*

Dissolve 50 mg of *boric acid* in *water* and dilute to 100.0 ml with *water*. Dilute 5.0 ml of the solution to 100.0 ml with *water*. Keep in a well-closed polyethylene container. In four polyethylene 25-ml flasks, place 0.5 g of the oral powder dissolved in 2 ml of *water* (solution A), 0.5 g of the oral powder dissolved in 1 ml of the reference solution and 1 ml of *water* (solution B), 1 ml of the reference solution and 1 ml of *water* (solution C) and 2 ml of *water* (solution D). To each flask, add 4 ml of *acetate-edetate buffer solution pH 5.5*. Mix and add 4 ml of freshly prepared *azomethine solution*. Mix and allow to stand for 1 hour. Measure the absorbance (2.4.7), of solutions A, B and C at 420 nm, using solution D as the compensation liquid. The test is not valid unless the absorbance of solution C is not less than 0.25. The absorbance of solution B is not less than twice that of solution A (9 ppm).

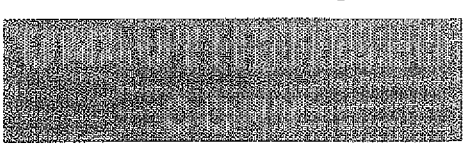
Lead. Not more than 0.5 ppm.

Determine by atomic absorption spectrometry (2.4.2).

Solvent mixture. Equal volumes of *dilute acetic acid* and *water*.

Test solution. Dissolve 20.0 g of the substance under examination in 100 ml of the solvent mixture. Add 2.0 ml of 1 per cent w/v solution of *ammonium pyrrolidinedithiocarbamate* and 10.0 ml of *methyl isobutyl ketone*, shake for few seconds protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solution (a). Dilute 0.5 ml of *lead standard solution (10 ppm Pb)* to 100 ml with the solvent mixture. Add 2.0 ml of a clear 1 per cent w/v solution of *ammonium pyrrolidinedithio-*



carbamate and 10.0 ml of *methyl isobutyl ketone*, shake for few seconds and protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solution (b). Dilute 1.0 ml of *lead standard solution (10 ppm Pb)* to 100 ml with the solvent mixture. Add 2.0 ml of a clear 1 per cent w/v solution of *ammonium pyrrolidinedithiocarbamate* and 10.0 ml of *methyl isobutyl ketone*, shake for few seconds and protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solution (c). Dilute 1.5 ml of *lead standard solution (10 ppm Pb)* to 100 ml with the solvent mixture. Add 2.0 ml of a clear 1 per cent w/v solution of *ammonium pyrrolidinedithiocarbamate* and 10.0 ml of *methyl isobutyl ketone*, shake for few seconds and protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Set the zero of the instrument using *methyl isobutyl ketone* treated as described for the test solution without the substance under examination. Measure the absorbance at 283.3 nm using a lead hollow-cathode lamp as source of radiation and an air-acetylene flame.

Water (2.3.43). Not more than 2.5 per cent, determined on 0.5 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Other tests. Comply with the tests stated under Oral Powders.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 1 g of the oral powder in 10 ml of *water*, add 12.5 ml of *acetonitrile* with gentle heating and dilute to 25.0 ml with *water*.

Reference solution (a). Dissolve 1 g of *lactulose IPRS* in 10 ml of *water*, add 12.5 ml of *acetonitrile* with gentle heating and dilute to 25.0 ml with *water*.

Reference solution (b). Add 47.5 ml of *acetonitrile* to 3 ml of reference solution (a) with gentle heating and dilute to 100.0 ml with *water*.

Reference solution (c). Dissolve 20 mg of *lactulose IPRS* and 20 mg of *epilactose IPRS* in 2 ml of *water*, add 2.5 ml of *acetonitrile* with gentle heating and dilute to 5.0 ml with *water*.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm followed by a stainless steel column 15 cm x 4.6 mm, both packed with aminopropylsilane bonded to porous silica (3 µm),
- column temperature: 38°,
- mobile phase: a mixture of 0.253 g of *sodium dihydrogen orthophosphate* in 220 ml of *water* and 780 ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- differencial refractometer detector maintained at a constant temperature,
- injection volume: 20 µl.

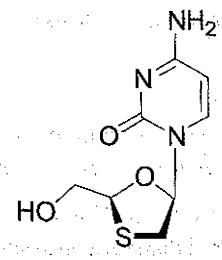
The relative retention time with reference to lactulose (retention time: about 18.3 minutes) for tagatose is about 0.38, for fructose is about 0.42, for galactose is about 0.57, for epilactose is about 0.90 and for lactose is about 1.17.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to lactulose and epilactose is not less than 1.3.

Inject reference solution (a) and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak.

Calculate the content of $C_{12}H_{22}O_{11}$.

Lamivudine



$C_8H_{11}N_3O_3S$ Mol. Wt. 229.3

Lamivudine is (2*R*,5*S*)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5yl]-2(1*H*)-pyrimidinone.

Lamivudine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_{11}N_3O_3S$, calculated on the anhydrous basis.

Category. Antiretroviral.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lamivudine IPRS* or with the reference spectrum of lamivudine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lamivudine in the chromatogram obtained with the reference solution.

C. Melting range (2.4.21). 172° to 178°.

Tests

Specific optical rotation (2.4.22). –144° to –136°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of substance under examination in 70 ml of the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *salicylic acid (lamivudine impurity C)* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.001 per cent w/v each of *lamivudine IPRS* and *salicylic acid* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 5 volumes of *methanol* and 95 volumes 0.19 per cent w/v of *ammonium acetate,* adjusted to pH 3.8 with *glacial acetic acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 277nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Lamivudine impurity E[1]	0.28	0.6
Lamivudine impurity F[2]	0.32	2.2
Lamivudine impurity A[3]	0.36	—
Lamivudine impurity B[4]	0.91	—
Lamivudine (Retention time: about 9 minutes)	—	—
Lamivudine impurity J[5]	1.45	2.2
Lamivudine impurity C[6]	2.32	—

[1]4-aminopyrimidin-2(1*H*)-one (cytosine),

[2]pyrimidine-2,4(1*H*,3*H*)-dione (uracil),

[3](2*RS*,5*SR*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid,

[4]4-amino-1-[(2*RS*,5*RS*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)- one ((±)-*trans*-lamivudine),

[5]1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidine-2,4(1*H*,3*H*)-dione,

[6]2-hydroxybenzenecarboxylic acid (salicylic acid).

Inject reference solution (c). Run the chromatogram twice the retention time of lamivudine. The order of elution is lamivudine and then, salicylic acid. The test is not valid unless the resolution between the peaks due to lamivudine and salicylic acid is not less than 10, the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 1.5 for lamivudine peak.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak due to lamivudine impurity A, is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any secondary peak due to lamivudine impurity B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the area of any secondary peak due to lamivudine impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent). Ignore any peak with an area less than 0.5 times the peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent determined on 2.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.025 per cent w/v solution of *lamivudine IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{11}N_3O_3S$.

Storage. Store protected from light and moisture.

Lamivudine Oral Solution

Lamivudine Oral Solution is a solution of Lamivudine in a suitable flavoured vehicle.

Lamivudine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamivudine, $C_8H_{11}N_3O_3S$.

Usual strengths. 25 mg in 5 ml; 50 mg in 5 ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *1-butanol*, 30 volumes of *heptane*, 30 volumes of *acetone* and 10 volumes of *strong ammonia solution*.

Test solution. Dilute the preparation under examination with *methanol* to obtain a solution containing 2 mg of lamivudine per ml.

Reference solution. A 0.2 per cent w/v solution of *lamivudine IPRS* in a mixture of 75 volumes *methanol* and 25 volumes of *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lamivudine in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.0 to 7.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately measured volume of the preparation under examination containing 50 mg of lamivudine to a 50-ml volumetric flask, add about 30 ml of a solution prepared by mixing 10 volumes of *acetonitrile* and 90 volumes of *0.1 M potassium dihydrogen phosphate*, the pH of which is adjusted to 3.0 with *dilute phosphoric acid* (solution A), mix with the aid of ultrasound for 5 minutes, dilute to volume with solution A and filter. Dilute 5 ml of the filtrate to 50 ml with solution A.

Reference solution. Weigh 25 mg of *lamivudine IPRS* and transfer to a 50-ml volumetric flask, dissolve and dilute to volume with solution A. Further dilute 5 ml of the solution to 250 ml with solution A.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: A. *0.05 M potassium dihydrogen phosphate*, adjusted to pH 3.0 with *orthophosphoric acid*,

B. *acetonitrile*,

- a gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	02
10	85	15
25	30	70
35	30	70
40	98	02
50	98	02

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 15,000 theoretical plates and the tailing factor is not more than 1.5.

Inject solution A and the test solution. Examine the chromatogram obtained with solution A for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution. Ignore any peaks due to preservatives also.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 1.0 per cent and the sum of the areas of all the secondary peaks should not be more than 2.0 per cent when calculated by percentage area normalisation.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the preparation under examination containing about 100 mg of lamivudine in a 100-ml volumetric flask, add about 50 ml of *water*, mix with the aid of ultrasound for 10 minutes, dilute to volume with *water*, mix and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with *water*.

Reference solution. Weigh 100 mg of *lamivudine IPRS* in a 100-ml volumetric flask, dissolve and dilute to volume with *water*. Dilute 5.0 ml of the solution to 50.0 ml with *water*.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: 80 volumes of *water* and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 3000 theoretical plates, the tailing factor is not more

than 2.0 and the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml (2.4.29) of the oral solution and calculate the content of $C_8H_{11}N_3O_3S$ weight in volume.

Lamivudine Tablets

Lamivudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamivudine, $C_8H_{11}N_3O_3S$.

Usual strengths. 100 mg; 150 mg; 300 mg.

Identification

A. When examined in the range 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in a mixture of 50 volumes of *water* and 50 volumes of *acetonitrile,* shows an absorption maximum at about 270 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lamivudine in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.01 M hydrochloric acid,*

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 270 nm (2.4.7). Calculate the content of $C_8H_{11}N_3O_3S$, in the medium from the absorbance obtained from a solution of known concentration of *lamivudine IPRS* in *0.01 M hydrochloric acid.*

Q. Not less than 70 per cent of the stated amount of $C_8H_{11}N_3O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 600 mg of lamivudine in 20 ml of *water*, with the aid of ultrasound. Add 20 ml of *acetonitrile,* mix with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with *water* and filter.

Reference solution. A 0.024 per cent w/v solution of *lamivudine IPRS* in a mixture of 80 volumes of *water* and 20 volumes of *acetonitrile.*

Chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution. The area of any secondary peak is not more than 1.0 per cent and the sum of the areas of all the secondary peaks is not more than 2.0 per cent calculated by area normalisation.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *water* and 20 volumes of *acetonitrile*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 300 mg of Lamivudine with the solvent mixture to have a final concentration of 0.024 per cent of Lamivudine. Disperse with the aid of ultrasound and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution. A 0.024 per cent w/v solution of *lamivudine IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 5 volumes of *methanol* and 95 volumes of a buffer prepared by dissolving 1.0 g of *ammonium acetate* and 1.0 ml of *glacial acetic acid* in sufficient *water* to make 1000 ml and adjusted to pH 3.8 with *glacial acetic acid,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{11}N_3O_3S$ in the tablets.

Storage. Store protected from moisture.

Lamivudine and Tenofovir Tablets

Lamivudine and Tenofovir Disoproxil Fumarate Tablets

Lamivudine and Tenofovir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, $C_8H_{11}N_3O_3S$ and tenofovir disoproxil fumarate, $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$.

Usual strengths. Lamivudine 300 mg and Tenofovir disoproxil fumarate 300 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. 5.0 ml, each of, a 0.16 per cent w/v solution of *lamivudine IPRS* and *tenofovir disoproxil fumarate IPRS* in *methanol*, diluted to 25.0 ml with the dissolution medium.

Use the chromatographic system given in the Assay.

Inject the reference solution. The resolution between the peaks due to lamivudine and tenofovir disoproxil is not less than 2.0.

Inject the reference solution and the test solution.

Calculate the contents of $C_8H_{11}N_3O_3S$ and $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ in the medium.

Q. Not less than 75 per cent of the stated amounts of $C_8H_{11}N_3O_3S$ and $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Buffer solution. Dissolve 1.9 g of *ammonium acetate* in 950 ml of *water*, adjusted to pH 3.8 with *glacial acetic acid* and dilute to 1000 ml of *water*.

Solvent mixture. 80 volumes of *water* and 20 volumes of *methanol*.

Test solution. Disperse a quantity of the powdered tablets containing 100 mg of Lamivudine in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A solution containing 0.00025 per cent w/v each of *lamivudine IPRS* and *tenofovir disoproxil fumarate IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as YMC-pack ODS-AQ),
- column temperature: 40°,
- mobile phase: A. a mixture of 95 volumes of buffer solution and 5 volumes of *methanol*,
  B. a mixture of 30 volumes of the buffer solution, 68 volumes of *methanol* and 2 volumes of *tertiary butanol*,
- a gradient programme using the conditions given below,
- flow rate: 1ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
10	100	0
30	10	90
42	10	90
47	0	100
52	0	100
57	100	0
65	100	0

Name	Relative retention time	Correction factor
Mono ester impurity[1*]	0.75	0.81
Mono POC dimer impurity[2*]	0.9	0.76
Tenofovir disoproxil (Retention time: about 32 minutes)	1.0	—
Mix dimer impurity[3*]	1.1	0.71
Carbonyl impurity[4*]	1.15	0.96
Tenofovir disoproxil dimer impurity[5*]	1.5	0.79
Lamivudine (Retention time is about 12 minutes)	1.0	—
Carboxylic acid impurity[6#]	0.38	0.93
Lamivudine diastereomer impurity[7#]	0.94	1.19

*Relative retention time with reference to tenofovir disoproxil peak.

#Relative retention time with reference to lamivudine peak.

[1]Carbonic acid [[[[(1R)-2-(6-Amino-9*H*-purin-9-yl)-1-methylethoxy] methyl]hydroxyphosphenyl]oxy]methyl-1-methylethylester,

[2]{[(propan-2-yloxy)carbonyl]oxy}methylhydrogen(*S*)-({[(2S)-1-(6[({[9-(5-hydroxy-2,11-dimethyl-5-oxido-9-oxo-3,6,8,10-tetraoxa-5λ5-phosphadodec-1-yl]-9*H*-purin-6-yl}amino}methyl}amino]-9*H*-purin-9-yl}propan-2-yl] methyl) phosphonate,

[3]{[(propan-2-yloxy)carbonyl]oxy}methylhydrogen(R)-({[(2S)-1-(6{[({9-[2,11-dimethyl-5-oxido-9-oxo-5-({[(propan-2-yloxy) carbonyl]oxy}methoxy-3,6,8,10-tetraoxa-5λ5-phosphadodec-1-yl]-9*H*-purin-6-yl}amino}methyl}amino]-9*H*-purin-9-yl}propan-2-yl] methyl) phosphonate,

[4]Bis{[isopropoxycarbonyl)oxy]methyl}[((1R)-2-{6-isopropoxy carbonyl)amino]-9*H*-purin-9-yl}-1-methyl ethoxymethyl] phosphonate,

[5]Tetrakis{[(isopropoxycarbonyl)oxy]methyl}[methylenebis(imino-9*H*-purine-6,9-diylpropane-1,2-diylpropane-1,2-diyloxymethylene)] bis (phosphonate),

[6]4-amino-2-oxo-pyrimidinyl-1,3-oxatiolane-2-carboxylic acid

[7](2*S-cis*)-(-)-1[(2*R*,5*R*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl] cytosisine.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 and 10000 theoretical plates for lamivudine and tenofovir disoproxil peak, respectively, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 10.0 per cent for both the peaks.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to mono ester impurity is not more than 6 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with the reference solution (1.5 per cent), the area of any peak corresponding to tenofovir disoproxil dimer impurity is not more than 0.8 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with the reference solution (0.2 per cent), the area of any peak corresponding to mono POC dimer impurity is not more than twice the area of the peak due to tenofovir disoproxil in the chromatogram obtained with the reference solution (0.5 per cent), the area of any peak corresponding to mix dimer impurity is not more than 3 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with the reference solution (0.75 per cent), the area of any peak corresponding to carboxylic acid impurity is not more than 1.2 times the area of the peak due to lamivudine in the chromatogram obtained with the reference solution (0.3 per cent), the area of any peak corresponding to lamivudine diastereomer impurity is not more than 0.8 times the area of the peak due to lamivudinein the chromatogram obtained with the reference solution (0.2 per cent), the area of any other secondary peak is not more than 0.8 times the area of the peak due to tenofovir disoproxil in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of all the secondary peaks is not more than 4.0 per cent. Ignore the peak due to fumaric acid with a retention time at about 3.5 minutes and any peak with an area less than 0.2 times the area of the tenofovir disoproxil peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Lamivudine, dissolve in 100.0 ml of the mobile phase and filter.

Reference solution. A 0.1 per cent w/v solution each of *lamivudine IPRS* and *tenofovir disoproxil fumarate IPRS* in the mobile phase. Dilute 20.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 7.8 g of *sodium dihydrogen orthophosphate dihydrate* in 1000 ml of *distilled water*, adding 1 ml of *triethylamine* and adjusted to pH 2.3 with *orthophosphoric acid* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the peaks due to lamivudine and tenofovir disoproxil is not less than 750 and 1500 theoretical plates respectively, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{11}N_3O_3S$ and $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ in the tablets.

Storage. Store protected from moisture, at temperature not exceeding 30°.

Lamivudine and Zidovudine Tablets

Lamivudine and Zidovudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, $C_8H_{11}N_3O_3S$ and zidovudine, $C_{10}H_{13}N_5O_4$.

Usual strength. Lamivudine, 150 mg and zidovudine, 300 mg.

Identification

In the Assay, the two principal peaks in the chromatogram obtained with the test solution correspond to the peaks due to lamivudine and zidovudine in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution. Dissolve an accurately weighed quantity of *lamivudine IPRS* and *zidovudine IPRS* in the mobile phase and dilute with the mobile phase to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 60 volumes of a buffer solution containing *0.1 M ammonium acetate* in 0.1 per cent *acetic acid* and 40 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections for each of the peaks corresponding to lamivudine and zidovudine is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the contents of $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$.

Q. Not less than 75 per cent of the stated amounts of $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 95 volumes of mobile phase A and 5 volumes of mobile phase B.

Test solution. Disperse a quantity of the powdered tablets containing 150 mg of Lamivudine in *water* with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with *water*; filter. Dilute 1.0 ml of the filtrate to 10.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.015 per cent w/v of *lamivudine IPRS* and 0.03 per cent w/v of *zidovudine IPRS* in the solvent mixture.

Reference solution (b). A 0.017 per cent w/v solution of *lamivudine resolution mixture B IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 1.95 g of *ammonium acetate* in 900 ml of *water*, adjusted to pH 4.0 with *glacial acetic acid* and dilute to 1000.0 ml with *water*,

  B. *methanol*,

  C. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Mobile phase C (per cent v/v)
0	95	5	0
15	95	5	0
30	70	30	0
38	70	30	0
38.1	0	0	100
45	0	0	100
45.1	95	5	0
60	95	5	0

Name	Relative retention time	Correction factor	Acceptance Criteria Not more than (per cent)
Lamivudine-(cytosine)[1,*]	0.11	—	—
Lamivudine-(uracil)[2,*]	0.14	—	—
Lamivudine-(carboxylic acid)[*]	0.17	—	0.3
Lamivudine-(S-sulphoxide)[3,*]	0.20	—	—
Lamivudine-(R-sulphoxide)[4,*]	0.22	—	—
Zidovudine impurity C[5]	0.27	0.59	1.5
Lamivudine diastereomer[6]	0.50	—	0.2
Lamivudine	0.52	—	—
Zidovudine-(thymidine)[7,*]	0.60	—	—
Lamivudine-(uracil derivative)[8,*]	0.70	—	—
Lamivudine-(salicylic acid)[9,*]	0.80	—	—
Zidovudine	1.0	—	—
Zidovudine impurity B[10,*]	1.1	—	—
Any other secondary impurity	—	—	0.1
Total lamivudine related impurities	—	—	0.6
Total zidovudine related impurities (The limit includes other impurities)	—	—	2.0

[*]These are the process related impurities, monitored in the drug substance,

[1]4-Aminopyrimidin-2(1*H*)-one,

[2]Pyrimidine-2,4(1*H*,3*H*)-dione,

[3]1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide,

[4]1-[(2*R*,3*S*,5*SS*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide,

[5]5-Methylpyrimidine-2,4(1*H*,3*H*)-dione,

[6]1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine,

[7][1-(2-Deoxy-α-d-ribofuranosyl)]thymine,

[8](2*R*,5*S*)1-[(2*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil,

[9]2-Hydroxybenzoic acid,

[10]3'-Chloro-3'-deoxythymidine.

Inject reference solution (a) and (b). The test is not valid unless the resolution between lamivudine diastereomer and lamivudine peaks is not less than 1.5 in the chromatogram obtained with reference solution (b) and the relative standard deviation of replicate injections is not more than 2.0 per cent for each component in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the percentage of each lamivudine related impurity in the portion of tablets taken:

$$\text{Result} = \frac{r_U}{r_T} \times 100$$

where, r_U = peak response of each lamivudine related impurity from the test solution,

r_T = sum of the peak responses of lamivudine and all lamivudine related impurities from the test solution.

Calculate the percentage of each zidovudine related impurity and other impurity in the portion of tablets taken:

$$\text{Result} = \frac{r_U}{r_T} \times c \times 100$$

where, r_U = peak response of each zidovudine related impurity and other secondary impurity from the test solution,

r_T = sum of the peak responses of zidovudine, all zidovudine related impurities and other impurities from the test solution,

c = correction factor.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder equivalent to the average weight of one tablet into a 200-ml volumetric flask. Add 100 ml of the mobile phase and disperse with the aid of ultrasound for about 15 minutes with occasional shaking to obtain a uniform dispersion. Cool to room temperature and dilute to volume with the mobile phase. Filter the solution through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute 5.0 ml of the filtrate to 25.0 ml with the mobile phase. Further dilute 5.0 ml to 50.0 ml with the mobile phase and mix.

Reference solution. Weigh 30 mg of *lamivudine IPRS* and 60 mg of *zidovudine IPRS*, transfer to a 100 ml volumetric flask, dissolve in the mobile phase and dilute to volume with the mobile phase. Further dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 60 volumes of a buffer solution containing *0.1 M ammonium acetate* in 0.1 per cent *acetic acid* and 40 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections for each of the peaks corresponding to lamivudine and zidovudine is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the contents of $C_8H_{11}N_3O_3S$ and $C_{10}H_{13}N_5O_4$ in the tablets.

Storage. Store protected from moisture.

Lamivudine, Nevirapine and Stavudine Dispersible Tablets

Lamivudine, Nevirapine and Stavudine Dispersible Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, $C_8H_{11}N_3O_3S$, nevirapine, $C_{15}H_{14}N_4O$ and stavudine, $C_{10}H_{12}N_2O_4$.

Usual strengths. Lamivudine 30 mg, Nevirapine 50 mg and Stavudine 6 mg; Lamivudine 60 mg, Nevirapine 100 mg and Stavudine 12 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *water* and 50 volumes of *methanol*.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution containing 0.02 per cent w/v of *stavudine IPRS*, 0.09 per cent w/v *lamivudine IPRS* and 0.15 per cent w/v of *nevirapine IPRS* dissolved in minimum quantity of *methanol* and makeup with solvent mixture. Dilute 5 ml of the solution to 100 ml with the dissolution medium.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1 g of *octane sulphonic acid sodium salt* and 0.68 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adding 1 ml of *triethylamine* and adjusted to pH 2.5 with *orthophosphoric acid* and 35 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency in not less than 2000 theoretical plates for lamivudine, the tailing factor is not more than 1.5 for each component and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$.

Q. Not less than 80 per cent of the stated amounts of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. 70 volumes of a solution containing 0.2 per cent v/v of *orthophosphoric acid* and 30 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 75 mg of Lamivudine, dissolve in the solvent mixture and dilute to 100.0 ml with the solvent mixture, filter.

Reference solution. Weigh 75 mg of *lamivudine IPRS*, 130 mg of *nevirapine IPRS* and 20 mg of *stavudine IPRS*, dissolve in 20 ml of *methanol* and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *methanol*,
  B. a buffer solution prepared by dissolving 1.925 g of *ammonium acetate* in 1000 ml of *water* and adjusted to pH 3.0 with *trifluoroacetic acid*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	5	95
15	15	85
30	45	55
40	60	40
48	60	40
51	5	95
60	5	95

Name	Relative retention time
Carboxylic acid	0.36
Thymine	0.42
Lamivudine	0.74
Stavudine	1.0
Nevirapine	2.19

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and tailing factor is not more than 1.5 for each component.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3 times the area of principal peak due to lamivudine in the chromatogram obtained with the reference solution (3.0 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of principal peak due to lamivudine in the chromatogram obtained with the reference solution (5.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described under Assay using the following solution as the test solution.

Test solution. Disperse one tablet in 20 ml of *water*, add 80 ml of the solvent mixture and mix with the aid of ultrasound and dilute to 250.0 ml with the solvent mixture, filter.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *methanol*.

Test solution. Weigh accurately a quantity of the powdered tablets containing 50 mg of Lamivudine, dissolve in 100.0 ml of solvent mixture. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture and filter.

Reference solution. A solution containing 0.10 per cent w/v of *lamivudine IPRS*, 0.175 per cent w/v *nevirapine IPRS* and 0.025 per cent w/v of *stavudine IPRS* dissolved in minimum quantity of *methanol* and makeup with solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a buffer solution prepared by dissolving 1 g of *octane sulphonic acid* and 0.68 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adding 1 ml of *triethylamine* and adjusted to pH 2.5 with *orthophosphoric acid* and 35 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency in not less than 2000 theoretical plates for lamivudine, the tailing factor is not more than 1.5 for each component and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 25°.

Lamivudine, Nevirapine and Stavudine Tablets

Lamivudine, Nevirapine and Stavudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lamivudine, $C_8H_{11}N_3O_3S$, nevirapine, $C_{15}H_{14}N_4O$ and stavudine, $C_{10}H_{12}N_2O_4$.

Usual strengths. Stavudine, 30 mg, Lamivudine, 150 mg and Nevirapine, 200 mg; Stavudine, 40 mg, Lamivudine, 150 mg and Nevirapine, 200 mg.

Identification

In the Assay, the three principal peaks in the chromatogram obtained with the test solution have retention times similar to those of the peaks due to lamivudine, nevirapine and stavudine in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Determine by liquid chromatography (2.4.14).

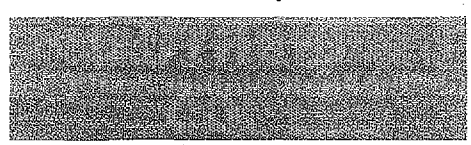
Test solution. Use the filtrate, dilute if necessary.

Reference solution. Weigh 150 mg of *lamivudine IPRS*, 200 mg of *nevirapine IPRS* and 30 mg of *stavudine IPRS* (if claim of stavudine per tablet is 30 mg) or 40 mg of *stavudine IPRS* (if claim of stavudine per tablet is 40 mg) and transfer to a 100-ml volumetric flask. Add about 20 ml of *methanol*, disperse with the aid of ultrasound to dissolve and dilute to volume with a solvent mixture of equal volumes of *methanol* and *water*. Dilute 5.0 ml of the solution to 50.0 ml with *0.01 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a degassed mixture of 35 volumes of *methanol* and 65 volumes of a buffer solution prepared by dissolving 0.68 g of *potassium dihydrogen phosphate* and 1.0 g of *sodium octanesulphonate* in 1000.0 ml of *water* to which 1 ml of *triethylamine* is added and adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 2000 theoretical plates, the tailing factor for the individual lamivudine, nevirapine and stavudine peaks is not more than 1.5 and the relative standard deviation for replicate injections of all the analyte peaks is not more than 1.0 per cent.

Inject the reference solution and the test solution.



Calculate the contents of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$ in the medium.

Q. Not less than 70 per cent of the stated amounts of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets (a minimum of 2 tablets should be powdered) containing 100 mg of nevirapine, transfer to a 200-ml volumetric flask and add about 150 ml of *water*. Disperse with the aid of ultrasound for 10 minutes with occasional shaking to obtain a uniform dispersion, cool to room temperature, dilute to volume with *water* and mix. Filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution (a). A solution containing 0.15 per cent w/v of *stavudine IPRS* and 0.015 per cent w/v of *thymine IPRS* in *water*.

Reference solution (b). Weigh 75 mg of *lamivudine IPRS* and about 100 mg of *nevirapine IPRS*, transfer to a 200-ml volumetric flask, add 20 ml of *methanol* and mix with the aid of ultrasound to dissolve. Add 10 ml of the test solution to this solution and make up to volume with *water* and filter.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *0.1 M ammonium acetate*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	05
05	95	05
25	20	80
30	20	80
31	95	05
35	95	05

Inject reference solution (a) and (b). The test is not valid unless the column efficiency for thymine, stavudine, lamivudine and nevirapine peaks is not less than 3000 theoretical plates and the tailing factor for the same peaks is not more than 2.0.

Inject the test solution and measure the peak responses of the major peaks due to lamivudine, nevirapine and stavudine. Calculate the amounts of related substances by the area normalisation method. The content of thymine is not greater than 3.0 per cent and that of any other impurity is not greater than 1.0 per cent. The sum of all the impurities is not greater than 3.5 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 150 mg of lamivudine to a 100-ml volumetric flask, add 20 ml of *methanol* and about 50 ml of a mixture of equal volumes of *water* and *methanol* and disperse with the aid of ultrasound for 5 minutes. Dilute suitably with the same solvent mixture to obtain a solution containing 0.15 mg of lamivudine per ml. Filter this solution through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution. A solution containing 0.015 per cent w/v of *lamivudine IPRS* and 0.02 per cent w/v of *nevirapine IPRS* and a concentration of *stavudine IPRS* similar to that of the concentration of stavudine in the test solution.

The chromatographic procedure may be carried out using the conditions described under Dissolution.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 2000 theoretical plates, the tailing factor for the individual peaks due to lamivudine, nevirapine and stavudine is not more than 1.5 and the relative standard deviation for replicate injections of all the analyte peaks is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the contents of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{12}N_2O_4$ in the tablets.

Storage. Store protected from moisture.

Lamivudine, Nevirapine and Zidovudine Paediatric Dispersible Tablets

Lamivudine, Nevirapine and Zidovudine Paediatric Dispersible Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of lamivudine, $C_8H_{11}N_3O_3S$, nevirapine, $C_{15}H_{14}N_4O$ and zidovudine, $C_{10}H_{13}N_5O_4$.

Usual strength. Lamivudine 30 mg, Nevirapine 50 mg, Zidovudine 60 mg.

Identification

A. In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

B. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2 volumes of *triethylamine*, 20 volumes of *isopropyl alcohol* and 80 volumes of *dichloromethane*.

Test solution. Weigh a quantity of the powdered tablet containing 15 mg of lamivudine, disperse in 10.0 ml of the *methanol* and filter.

Reference solution (a). A 0.15 per cent w/v solution of *lamivudine IPRS* in *methanol*.

Reference solution (b). A 0.2 per cent w/v solution of *nevirapine IPRS* in *methanol*.

Reference solution (c). A 0.3 per cent w/v solution of *zidovudine IPRS* in *methanol*.

Reference solution (d). A mixture of 1.0 ml each of reference solution (a), (b) and (c) diluted to 10.0 ml with *methanol*.

Apply to the plate 5 µl of the test solution and reference solution (d). Allow the mobile phase to raise 12 cm. Dry the plate in air and examine under the ultraviolet light at 254 nm. The principal spots in the chromatogram obtained with the test solution correspond to that in the chromatogram obtained with reference solution (d).

Tests

Disintigration (2.5.1). Not more than 3 minutes.

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *methanol*.

Test solution. Use the filtrate, dilute if necessary.

Reference solution. Dissolve 34 mg of *lamivudine IPRS*, 55 mg of *nevirapine IPRS* and 67 mg of *zidovudine IPRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with dissolution medium.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- sample temperature: 5°,
- mobile phase: a mixture of 35 volumes of *methanol* and 65 volumes of a buffer solution prepared by dissolving 0.68 g of *potassium dihydrogen phosphate* and 1 g of *sodium octanesulphonate* in 1000.0 ml of *water* to which 1.0 ml of *triethylamine* is added and adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume: 10 µl.

Inject the reference solution and the test solution.

Calculate the contents of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{13}N_5O_4$ in the medium.

Q. Not less than 75 per cent of the stated amounts of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{13}N_5O_4$.

Related substances. Determine by liquid chromatography (2.4.14)

Solvent mixture. 60 volumes of *water* and 40 volumes of *methanol*.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Lamivudine, in the solvent mixture and dilute to 100.0 ml with the solvent mixture, filter.

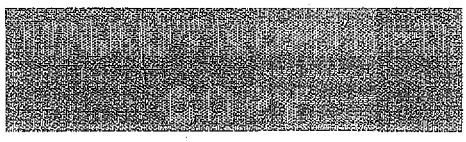
Reference solution (a). A solution containing 0.0001 per cent w/v of *lamivudine diastereomer IPRS* , 0.001 per cent w/v of *zidovudine impurity B IPRS*, 0.05 per cent w/v of *lamivudine IPRS* and 0.1 per cent w/v of *zidovudine IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.03 per cent w/v of *lamivudine IPRS*, 0.04 per cent w/v *of nevirapine IPRS* and 0.04 per cent w/v of *zidovudine IPRS* in the solvent mixture.

Reference solution (c). Dissolve 4 mg of *nevirapine impurity A IPRS* in 40 ml of *methanol* with the aid of ultrasound and dilute to 100.0 ml with *water*.

Reference solution (d). Dilute 5.0 ml of reference solution (b) and 5.0 ml of reference solution (c) to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Prontosil C18H),
- sample temperature: 5°,
- mobile phase: A. a mixture of 100 volumes of 0.23 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 3.5 with *orthophosphoric acid* and 0.2 volume of *methanol*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume: 20 µl.



Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	99	1
10	99	1
20	90	10
25	85	15
42	70	30
55	70	30
57	99	1
65	99	1

Name	Relative retention time	Correction factor
Lamivudine impurities		
Carboxylic acid impurity[1]	0.23	2.33*
Lamivudine diastereomer[2]	0.57	1.43*
Nevirapine impurities		
Nevirapine impurity A[3]	1.45	—
Nevirapine impurity B[4]	1.22	0.83#
Nevirapine impurity C[5]	1.67	1.03#
Zidovudine impurities		
Zidovudine impurity A[6]	0.76	0.86ϕ
Zidovudine	1.0	—
Zidovudine impurity B[7]	1.02	1.06ϕ
Zidovudine impurity C[8]	0.39	0.86ϕ
Thymidine[9]	0.66	0.93ϕ
Zidovudine threo isomer[10]	0.96	1.06ϕ
Zidovudine thymidine adduct[11]	1.28	0.98ϕ

*Correction factor with respect to Lamivudine

#Correction factor with respect to Nevirapine impurity A

ϕCorrection factor with respect to Zidovudine

[1]4-amino-2-oxo-pyrimidinyl-1,3-oxoathiolane-2-carboxylic acid,

[2] (2S-*cis*)-(-)-1-[(2R, 5R)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine,

[3]5,11-dihydro-6*H*-11-ethyl-4-methyl-dipyrido(3,2-b:2',3'-e)(1,4)diazepin-6-one,

[4](5,11-dihydro-4-methyl-6*H*-dipyrido(3,2-b:2',3'-e)(1,4)diazepin-6-one),

[5]5,11-dihydro-4-methyl-6*H*-11-propyl-dipyrido(3,2-b:2',3'-e)(1,4)diazepin-6-one,

[6]3'-azido-3'deoxy-3'-Azido-3'deoxythymidine; stavudine,

[7]3'-chloro-3'deoxythymidine,

[8]2,4-dihydroxy-5-methy pyrimidine; thymine,

[9]1-(2-deoxy-f"-D-ribifuranosyl)-5-methyl uracil,

[10]1'-(3-azido-2,3-dideoxy-f"-D-threo-pentafuranosyl)-thymidine,

[11]1-(3-(3-(3-azido-2,3-dideoxy-pentofuranosyl))-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1-yl)-2,3-dideoxypentofuranosyl)-5methylpyrimidine-2,4-dione.

The retention time of lamivudine, zidovudine, nevirapine and nevirapine impurity A peaks are about 18.5 minutes, 31 minutes, 40.6 minutes and 45 minutes respectively.

Inject reference solution (a) and (d). The test is not valid unless the resolution between the peaks due to lamivudine diastereomer and lamivudine is not less than 1.5 and between zidovudine and zidovudine impurity B is not less than 1.5 in the chromatogram obtained with reference solution (a), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent, for the peaks due to lamivudine, nevirapine related compound A and zidovudine in the chromatogram obtained with reference solution (d).

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to carboxylic acid impurity, lamivudine distereomer, each of, is not more than 1.66 times the area of the peak due to lamivudine in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of any peak corresponding to nevirapine impurity A, nevirapine impurity B and nevirapine impurity C, each of, is not more than 1.25 times the area of peak due to nevirapine impurity A in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of any peak corresponding to zidovudine impurity A (stavudine), zidovudine impurity B, thymidine, zidovudine threo isomers and zidovudine thymidine adduct, each of, is not more than 1.25 times the area of the peak due to zidovudine in the chromatogram obtained with reference solution (d) (0.5 per cent), the area of any peak corresponding to zidovudine impurity C (thymine) is not more than 2.5 times the area of the peak due to zidovudine in the chromatogram obtained with reference solution (d) (1.0 per cent), the area of any other secondary peak is not more than 1.66 times the area of the peak due to lamivudine in the chromatogram obtained with reference solution (d) (0.5 per cent). The sum of all the impurities is not more than 2.5 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *0.01 M hydrochloric acid* and 50 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 300 mg of lamivudine, disperse in the solvent mixture and dilute to 500.0 ml with the solvent mixture, filter. Dilute 5.0 ml of the solution to 20.0 ml with the solvent mixture.

Reference solution. Dissolve 75 mg of *lamivudine IPRS*, 125 mg of *nevirapine IPRS* and 150 mg of *zidovudine IPRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture.

Use chromatographic system as described under Dissolution.

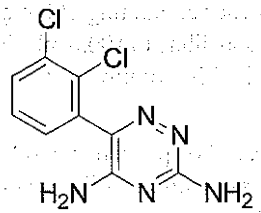
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the reference solution and the test solution.

Calculate the contents of $C_8H_{11}N_3O_3S$, $C_{15}H_{14}N_4O$ and $C_{10}H_{13}N_5O_4$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Lamotrigine



$C_9H_7Cl_2N_5$ Mol. Wt. 256.1

Lamotrigine is 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine.

Lamotrigine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_9H_7Cl_2N_5$, calculated on the dried basis.

Category. Anticonvulsant.

Description. A white to off - white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lamotrigine IPRS* or with the reference spectrum of lamotrigine.

B. When examined in the range 210 to 360 nm (2.4.7), a 0.0025 per cent w/v solution in *methanol* exhibits a maximum at about 309 nm.

C. In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of mobile phase A and 20 volumes of mobile phase B.

Test solution. Dissolve 100 mg of the substance under examination in 100 ml of solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *lamotrigine IPRS* in solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (*5* µm),
- mobile phase: A. 0.174 per cent w/v solution of *dipotassium hydrogen phosphate*, adjusted to pH 7.5 with *orthophosphoric acid* and filter,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
15	25	75
30	25	75
35	80	20

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 2 times the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°, under vacuum, for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100 ml of mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with mobile phase.

Reference solution. A 0.02 per cent w/v solution of *lamotrigine IPRS* in mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of 0.408 per cent w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 7.0 with *dilute potassium hydroxide solution*.
- flow rate:1.2 ml per minute,
- spectrophotometer set at 308 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_7Cl_2N_5$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Lamotrigine Dispersible Tablets

Lamotrigine Dispersible Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamotrigine, $C_9H_7Cl_2N_5$.

Usual strengths. 25 mg; 50 mg; 100 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. Dissolve an accurately weighed quantity of *lamotrigine IPRS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of a 0.408 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted to pH 7.0 with *potassium hydroxide*, 30 volumes of *acetonitrile*
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 308 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_7Cl_2N_5$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_9H_7Cl_2N_5$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 200 mg of Lamotrigine in 10 ml of *methanol* and dilute to 100.0 ml with the mobile phase, filter. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *lamotrigine IPRS*, dissolved in *methanol* and diluted with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of a 0.408 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted to pH 7.0 with *potassium hydroxide*, 30 volumes of *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 308 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_9H_7Cl_2N_5$.

Storage. Store protected from light and moisture.

Labelling. The label states that the tablets should be dispersed in water immediately before use.

Lamotrigine Prolonged-release Tablets

Lamotrigine Sustained-release Tablets; Lamotrigine Extended-release Tablets

Lamotrigine Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as

the dissolution profile of the products of different manufacturers may not be the same.

Lamotrigine Prolonged-release Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lamotrigine, $C_9H_7Cl_2N_5$.

Usual strengths. 50 mg; 100 mg; 200 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2). Complies with the test stated under tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *methanol* and 40 volumes of *water*.

Test solution. Weigh and transfer 5 tablets in to suitable volumetric flask, add *methanol* to 10 per cent of the volume of the flask and sonicate to disperse. Further add solvent mixture to 75 per cent of the volume of the flask and sonicate for 60 minutes in cool *water* with occasional shaking. Make up the volume with the solvent mixture. Centrifuge and dilute the supernatant liquid with the solvent mixture to prepare a 0.01per cent w/v solution.

Reference solution. A 0.0001 per cent w/v solution of *lamotrigine IPRS*, dissolved in minimum quantity of *methanol* and diluted with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *methanol* and 0.01 volume of *triethylamine*, adjusted to pH 7.0 with *10 per cent v/v* solution of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 309 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent) and sum of the areas of all the secondary peaks is not more than 1.5 times the area of the peak in the chromatogram obtained with the reference solution (1.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *methanol* and 40 volumes of *water*.

Test solution. Weigh and transfer 5 tablets in to suitable volumetric flask, add *methanol* to 10 per cent of the volume of the flask and sonicate to disperse. Further add solvent mixture to 75 per cent of the volume of the flask and sonicate for 60 minutes in cool *water* with ocassional shaking. Make up the volume with the solvent mixture. Centrifuge at 3500 rpm for 15 minutes. Dilute to obtain a solution of 0.01 per cent with the solvent mixture.

Reference solution. Dissolve 50 mg of *lamotrigine IPRS* in 10.0 ml of *methanol,* sonicate to dissolve and dilute to 100.0 ml with the solvent mixture. Further dilute the solution with the solvent mixture to obtain a solution of 0.01 per cent.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *methanol* and 0.01 volumes of *triethylamine*, adjusted to pH 7.0 with 10 per cent v/v solution of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 309 nm,
- injection volume: 20 µl.

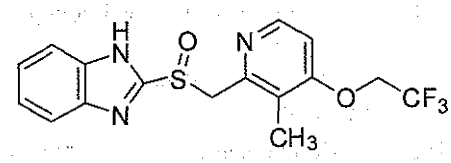
Inject the reference solution. The test is not valid unless the theoretical plates is not less than 3000 and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{72}N_2O_{12}$ in the tablets.

Storage. Store protected from light.

Lansoprazole



$C_{16}H_{14}F_3N_3O_2S$ Mol. Wt. 369.4

Lansoprazole is (*RS*)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulphinyl]-1*H*-benzimidazole.

Lansoprazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{14}F_3N_3O_2S$, calculated on the anhydrous basis.

Category. Antiulcer.

Description. A white to brownish-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lansoprazole IPRS* or with the reference spectrum of lansoprazole.

B. When examined in the range of 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows maxima at the same wavelength obtained with the solution having same concentration of the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use the solutions at or below 5°.

Solvent mixture. 75 volumes of *0.1 M sodium hydroxide solution* and 25 volumes of *methanol.*

Test solution. Dissolve about 125 mg of the substance under examination in 50 ml of *methanol.* Dilute 1.0 ml of the solution to 10.0 ml with solvent mixture.

Reference solution (a). A solution containing 5 mg each of *lansoprazole IPRS* and *lansoprazole impurity A IPRS* in 200 ml of *methanol.* Dilute 1 ml of the solution to 10 ml with solvent mixture.

Reference solution (b). A 0.0025 per cent w/v solution each of *lansoprazole IPRS* and *lansoprazole impurity B IPRS* in *methanol.* Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *water,*
  B. a mixture of 80 volumes of *acetonitrile,* 20 volumes of *water* and 0.5 volume of *triethylamine,* adjusted to pH 7.0 with *orthophosphoric acid,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 40 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
40	20	80
50	20	80
51	90	10
60	90	10

Name	Relative retention time	Correction factor
Lansoprazole *N*-oxide[1]	0.8	0.77
Lansoprazole	1.0	—
Lansoprazole impurity A[2]	1.1	1.22
Lansoprazole impurity B[3]	1.2	—

[1][[(1H -Benzimidazole-2-yl)sulphinyl]methyl]-3-methyl-4-(2,2,2-trifluoroethoxy)-pyridine 1-oxide,

[2]2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl] sulphonyl] benzimidazole,

[3]2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-pyridin-2-yl]methyl] sulphanyl]-1H –benzimidazole.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to lansoprazole and lansoprazole impurity A is not less than 6 and the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to lansoprazole impurity A is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent), the area of each peak due to lansoprazole *N*-oxide and lansoprazole impurity B is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all the secondary peaks is not more than 0.6 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.6 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.1 per cent, determined on a 1.0 g in a mixture of 90 volumes of *pyridine* and 10 volumes of *ethylene glycol.*

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water,* 40 volumes of *acetonitrile* and 1 volume of *triethylamine,* adjusted to pH 10.0 with *orthophosphoric acid.*

Internal standard solution. A 0.25 per cent w/v solution of *4-ethoxyacetophenone* in solvent mixture.

Test solution. Dissolve about 50 mg of the substance under examination in 10.0 ml of internal standard solution. Dilute 1.0 ml of the solution to 50 ml with solvent mixture.

Reference solution (a). A solution containing 0.01 per cent w/v each of *lansoprazole IPRS* and *lansoprazole impurity A IPRS* in the solvent mixture.

Reference solution (b). A 0.5 per cent w/v solution of *lansoprazole IPRS* in internal standard solution. Dilute 1.0 ml of the solution to 50.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *acetonitrile* and 1 volume of *triethylamine*, adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due lansoprazole and lansoprazole impurity A is not less than 5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{16}H_{14}F_3N_3O_2S$.

Storage. Store protected from light and moisture.

Lansoprazole Gastro-resistant Capsules

Lansoprazole Gastro-resistant Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$. They are made gastro-resistant by enteric-coating or by other means.

Usual strengths. 15 mg; 30 mg.

Identification

A. Shake the contents of Capsules containing about 5 mg of Lansoprazole in 5 ml of *methanol* and centrifuge. To 0.1 ml of the supernatant, add 10 ml of *methanol* and examine in the range 200 to 400 nm (2.4.7). The spectra obtained with the test solution exhibits the maxima at the same wavelength with that of the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

A. Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 60 minutes.

Withdraw 25 ml of the medium and proceed as directed for test solution in the test B, leaving the remaining 475 ml in the vessel for use in the test B and measure the absorbance of the filtrate, suitably diluted if necessary with dissolution medium at 306 nm (2.4.7). Calculate the content of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$ in the medium from the absorbance obtained from a solution of *lansoprazole IPRS* having a known concentration of about 8.0 per cent of the stated amount of lansoprazole dissolved per 500 ml of test A medium.

Complies with the acceptance criteria given under acid stage.

B. Apparatus No. 2 (Paddle),

Medium. 500 ml of buffer solution prepared by dissolving 16.35 g of *monobasic sodium phosphate*, 7.05 g of *sodium hydroxide* and 3 g of *sodium dodecyl sulphate* in 1000 ml of *water*,
Speed and time. 75 rpm and 60 minutes.

Add 425 ml of buffer solution to the remaining 475 ml of solution in each vessel from the test A. Adjusted to pH 6.8 with either *orthophosphoric acid* or *sodium hydroxide*, filter and measure the absorbances at 286 nm and 650 nm. Calculate the content of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$ in the medium from the difference between the absorbances at 286 nm and 650 nm obtained from a solution of *lansoprazole IPRS* having a known concentration of about 70 per cent of the stated amount of lansoprazole dissolved in 900 ml of buffer stage medium.

Q. Not less than 80 per cent of the stated amount of $C_{16}H_{14}F_3N_3O_2S$.

NOTE — A volume of methanol not to exceed 0.5 per cent of the total volume of standard solution may be used to dissolve Lansoprazole IPRS prior to dilution with acid stage medium and the amount of methanol not to exceed 2.0 per cent of the total volume of standard solution may be used to dissolve Lasoprazole IPRS prior to dissolution with buffer stage medium.

Uniformity of content. Shake the content of 1 Capsule with 30 ml of *0.1 M sodium hydroxide* in a 100-ml volumetric flask and sonicate to disintegrate. Dilute to volume with *acetonitrile*, centrifuge and filter. Dilute a volume of the filtrate with a mixture of 7 volumes of *acetonitrile* and 3 volumes of *0.1 M sodium hydroxide* to obtain a solution containing about 0.012 mg of lansoprazole per ml and measure the absorbance at about 294 nm.

Calculate the content of lansoprazole, $C_{16}H_{14}F_3N_3O_2S$ by using a 0.0012 per cent w/v solution of *lansoprazole IPRS* in a mixture of 7 volumes of *acetonitrile* and 3 volumes of *0.1 M sodium hydroxide*.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1 g of the contents of capsules by drying in an oven at 60° in vacuum over *phosphorus pentoxide*, at a pressure not exceeding 5 mm of Hg.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 60 volumes of *water*, 40 volumes of *acetonitrile* and 1 volume of *triethylamine*, adjusted to pH 10.0 with *orthophosphoric acid*.

Internal standard solution. A 0.75 per cent w/v solution of *4'-ethoxyacetophenone* in *acetonitrile*.

Test solution. Shake the contents of 10 Capsules containing about 300 mg of Lansoprazole with 60 ml of *0.1 M sodium hydroxide* in a 300-ml conical flask and sonicate until completely disintegrated. Add 20.0 ml of *acetonitrile* and 20 ml of internal standard solution, shake well and centrifuge. Dilute a volume of supernatant with solvent mixture to obtain a concentration of 0.1 mg of lansoprazole per ml and filter.

Reference solution (a). A solution containing 0.01 per cent w/v each of *lansoprazole IPRS* and *2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfonyl]benzimidazole IPRS (lansoprazole impurity A IPRS)* in the solvent mixture.

Reference solution (b). A 0.3 per cent w/v solution of *lansoprazole IPRS* in a mixture of 3 volumes of *0.1 M sodium hydroxide* and 2 volumes of *acetonitrile*. To 25.0 ml of the solution, add 5.0 ml of internal standard solution and dilute to 50.0 ml with the solvent mixture. Dilute this solution with the solvent mixture to obtain a solution containing 0.01 per cent w/v of Lansoprazole.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *water*, 40 volumes of *acetonitrile* and 1 volume of *triethylamine*, adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume: 10 µl.

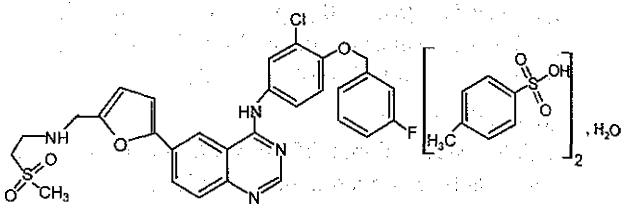
Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to lansoprazole and lansoprazole impurity A is not less than 5.0 in reference solution (a). The relative standard deviation for replicate injections is not more than 2.0 per cent in reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_{16}H_{14}F_3N_3O_2S$ in the Capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Lapatinib Ditosylate



$C_{29}H_{26}ClFN_4O_4S,2C_7H_8O_3S$ (anhydrous) Mol. Wt. 925.5

$C_{29}H_{26}ClFN_4O_4S,2C_7H_8O_3S,H_2O$ (monohydrate) Mol. Wt. 943.5

Lapatinib Ditosylate is *N*-[3-chloro-4-[(3-fluorophenyl) methoxy]phenyl]-6- [5-[(2-methylsulfonyl ethylamino)methyl]-2-furanyl]-4-quinazolinamine ditosylate.

Lapatinib Ditosylate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{29}H_{26}C1FN_4O_4S, 2C_7H_8O_3S$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lapatinib ditosylate IPRS* or with the reference spectrum of lapatinib ditosylate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

p-Toluenesulphonic acid. Not less than 36.1 per cent w/w and not more than 38.0 per cent w/w, calculated on anhydrous basis.

Dissolve 0.4 g of the substance under examination in 60 ml of *methanol* and 10 ml of *water*. Titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01722 g of p-toluenesulphonic acid.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *methanol*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution. A solution containing 0.001 per cent w/v each of *lapatinib ditosylate IPRS* and *lapatinib impurity B IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column of 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 2.72 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 7.0 with *triethylamine*,

  B: a mixture of 80 volumes of *acetonitrile* and 20 volumes *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	45	55
8	45	55
25	30	70
45	30	70
50	45	55
60	45	55

Name	Relative retention time
Lapatinib impurity B[1]	0.7
Lapatinib	1.0
Lapatinib impurity A[2]	1.4
Lapatinib impurity C[3]	1.3

[1]N-(4-(3-fluorobenzyloxy)phenyl)-6-(5-((2-methylsulfonyl) ethylamino)methyl)furan-2-l) quinazolin-4-amine-bis-4-methylbenzene sulfonate,

[2]5-(4-[3-chloro-4-(3-fluorobenzyloxy)amino]-6-quinazolinyl)-furan-2-carbaldehyde,

[3]4-(3-chloro-4-(3-fluorobenzyloxy)-phenylamino)-6-(5-hydroxy-methyl-furan-2-yl)quinazoline.

Inject the reference solution. The test is not valid unless the resolution between lapatinib impurity B and lapatinib is not less than 2.0, the column efficiency of the principal peak is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore the peak due to tosylate at relative retention time of about 0.14.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent (for anhydrous form) and 1.6 per cent to 2.5 per cent (for monohydrate form), determined on 0.5g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *lapatinib ditosylate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column of 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 7.0 with *triethylamine* and 65 volumes of a mixture of 80 ml of *acetonitrile* and 20 ml of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{26}C1FN_4O_4S, 2C_7H_8O_3S$.

Storage. Store protected from light.

Lapatinib Tablets

Lapatinib Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lapatinib, $C_{29}H_{26}ClFN_4O_4S$.

Usual strength. 250 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid* containing 2 per cent of *Tween 80*,
Speed and time. 55 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *acetonitrile*.

Test solution. Dilute 2 ml of the filtrate to 25 ml with the solvent mixture.

Reference solution. A 0.0036 per cent w/v solution of *lapatinib ditosylate IPRS* in the solvent mixture.

Use chromatographic system as described under Assay.

Q. Not less than 75 per cent of the stated amount of $C_{29}H_{26}ClFN_4O_4S$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *methanol*.

Test solution. Disperse a quantity of powdered tablets containing 100 mg of lapatinib in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.00016 per cent w/v solution of *lapatinib ditosylate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column, 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: A. a mixture of 98 volumes of buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.0 with *triethylamine* and 2 volumes of *methanol*,
  B: a mixture of 80 volumes of *acetonitrile* and 20 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	50	50
8	50	50
40	40	60
50	20	80
55	20	80
60	50	50
70	50	50

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 15 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent). Ignore the peak due to tosylate at relative retention time of about 0.14.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *methanol*.

Test solution (a). Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.25 g of lapatinib in the solvent mixture and dilute to 250.0 ml with the solvent mixture.

Test solution (b). Dilute 2.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution. A 0.00325 per cent w/v solution of *lapatinib ditosylate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 530 volumes of a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* in 1000 ml *water*, adjusted to pH 3.0 with *orthophosphoric acid*, 395 volumes of *acetonitrile* and 75 volumes of *tetrahydrofuran*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl.

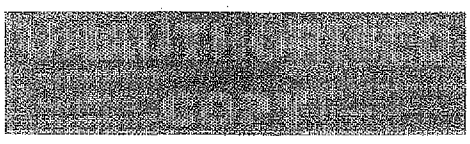
Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and test solution (b).

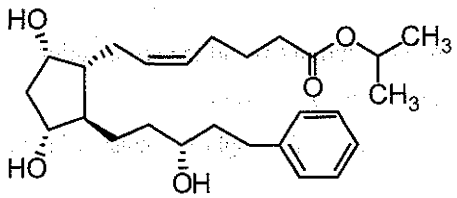
Calculate the content of $C_{29}H_{26}ClFN_4O_4S$ in the tablets.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of Lapatinib.



Latanoprost



$C_{26}H_{40}O_5$ Mol. Wt. 432.6

Latanoprost is isopropyl (*Z*)-7-[(1*R*, 2*R*, 3*R*, 5*S*)-3,5-dihydroxy-2-[(*R*)-3-hydroxy-5-phenylpentyl]cyclopentyl]-5-heptenoate.

Latanoprost contains not less than 94.0 per cent and not more than 102.0 per cent of $C_{26}H_{40}O_5$, calculated on the anhydrous basis.

Category. Antiglucoma.

Description. A colourless to slightly yellow oil.

Identification

NOTE — Wear protective glasses and gloves while handling the material. Avoid contact during pregnancy or while nursing.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *latanoprost IPRS* or with the reference spectrum of latanoprost.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Specific optical rotation (2.4.22). + 31.0° to + 38.0°, determined in a 1.0 per cent w/v solution of latanoprost in *acetonitrile.*

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay using the following modifications.

Solvent mixture. 80 volumes of *hexane* and 20 volumes of *ethanol.*

Reference solution (b). A 0.004 per cent w/v solution of *latanoprost IPRS* in the solvent mixture.

Name	Relative retention time	Correction factor
Isopropyl diphenyl phosphoryl pentanoate[1]	0.79	0.4
Latanoprost impurity B[2]	0.89	—
Latanoprost	1.0	—
Latanoprost impurity A[3]	1.1	—

[1]isopropyl 5-(diphenylphosphoryl) pentanoate,

[2]isopropyl (Z)-7-[(1R, 2R, 3R, 5S)-3, 5-dihydroxy-2-[(3S)-3-hydroxy-5-phenylpentyl] cyclopentyl]-5-heptenoate,

[3]isopropyl (E)-7-[(1R, 2R, 3R, 5S)-3, 5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl] cyclopentyl]-5-heptenoate.

Inject reference solution (a).The test is not valid unless the resolution between the peaks due to latanoprost and latanoprost impurity A is not less than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to isopropyl diphenylphosphoryl pentanoate is not more than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any peak corresponding to latanoprost impurity A is not more than 1.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.5 per cent), the area of any peak corresponding to latanoprost impurity B is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than 0.05 times the area of the principal peak obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks excluding latanoprost impurity A and impurity B is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with the area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Latanoprost impurity E. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of *acetonitrile* and 70 volumes of *water.*

Test solution. Dissolve 100 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.0001 per cent w/v solution of *latanoprost impurity E IPRS ((Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl]-5-heptanoic acid IPRS)* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 60°,
- mobile phase: A. a mixture of 300 volumes of *acetonitrile*, 1 volume of *orthophosphoric acid* and 700 volumes of *water*,
  B. a mixture of 800 volumes of *acetonitrile*, 1 volume of *orthophosphoric acid* and 200 volumes of *water*,
- a gradient programme using the conditions given below,

– flow rate: 1 ml per minute,
– spectrophotometer set at 200 nm,
– injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
9	100	0
10	0	100
15	0	100
16	100	0
21	100	0

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to latanoprost impurity E is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on a 10.0 per cent solution of latanoprost in *ethyl acetate*. Carry out blank determination.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 200 mg of substance under examination in 20 ml of *ethanol* and dilute to 100.0 ml with *hexane*.

Reference solution (a). Dissolve weighed quantities of *latanoprost IPRS* and *latanoprost impurity A IPRS* in *ethanol* (equivalent to 20 per cent of the final volume) and dilute with *hexane* to obtain a solution having concentration equivalent to 0.2 per cent of latanoprost and 0.002 per cent of latanoprost impurity A.

Reference solution (b). Dissolve 20 mg of *latanoprost IPRS* in 2 ml of *ethanol* and dilute to 10.0 ml with *hexane*.

Chromatographic system
– a stainless steel column 25 cm x 4.0 mm, packed with porous silica (5 µm),
– mobile phase: a mixture of 94 volumes of *hexane* and 6 volumes of *ethanol*,
– flow rate: 1 ml per minute,
– spectrophotometer set at 210 nm,
– injection volume: 10 µl.

The relative retention time with reference to latanoprost for latanoprost impurity A is about 1.1.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to latanoprost and latanoprost impurity A is not less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{26}H_{40}O_5$.

Storage. Store protected from light and moisture in a refrigerator or freezer.

Latanoprost Eye Drops

Latanoprost Eye Drops is a sterile solution of Latanoprost in Purified Water.

Latanoprost Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Latanoprost, $C_{26}H_{40}O_5$.

Usual strength. 0.005 per cent w/v.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.4 to 7.0.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay.

Inject the test solution. The area of any peak due to latanoprost free acid and 15-keto latanoprost is not more than 0.5 per cent. The area of any other secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization. Ignore peak due to impurity at relative retention time about 1.03 and the peaks having an area less than 0.1 per cent. Ignore all the peaks eluted till the retention time of 6 minutes.

Name	Relative retention time
Latanoprost free acid	0.40
Latanoprost	1.0
15-keto Latanoprost	1.21

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of equal volumes of *water* and *acetonitrile*.

Test solution. Prepare a composite solution by pooling the contents of 10 containers of eye drops into a suitable vessel. Weigh and transfer composite sample containing 0.35 mg of Latanoprost, into 10.0 ml volumetric flask. Add 2 ml of *acetonitrile*, sonicate for 5 minutes and dilute to volume with *solvent mixture.*

Reference solution. Dissolve 22 mg of *latanoprost IPRS* in 30.0 ml of the *acetonitrile* and dilute to 50.0 ml with the *acetonitrile*. Dilute 2.0 ml of the solution to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 58°,
- sample temperature: 5°,
- mobile phase: A. a mixture of 30 volumes of *acetonitrile*, 4 volumes of *isopropyl alcohol* and 66 volumes of a buffer solution prepared by dissolving 0.3 g of *sodium dihydrogen orthophosphate monohydrate* in 1000 ml *water*, adjusted to pH 5.0 with *orthophosphoric acid* or *dilute sodium hydroxide solution* and filter.

  B. a mixture of 16 volumes of *water*, 4 volumes of *isopropyl alcohol* and 80 volumes of *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
8	95	5
10	80	20
30	80	20
35	70	30
40	50	50
40.1	100	0
50	100	0

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml of the sample (2.4.29) and calculate the content of $C_{26}H_{40}O_5$ as weight in volume.

Storage. Store at a temperature between 2° to 8°.

Latanoprost and Timolol Ophthalmic Solution

Latanoprost and Timolol Maleate Ophthalmic Solution

Latanoprost and Timolol Ophthalmic Solution is a sterile solution of Latanoprost and Timolol Maleate in a suitable aqueous vehicle.

Latanoprost and Timolol Ophthalmic Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of latanoprost, $C_{26}H_{40}O_5$ and timolol, $C_{13}H_{24}N_4O_3S$.

Usual strength. Latanoprost, 50 mcg and Timolol Maleate equivalent to Timolol 5 mg per ml.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.5 to 6.5.

Related substances. Determine by liquid chromatography (2.4.14).

For Timolol —

Buffer solution. A buffer solution prepared by dissolving 1.7 g of *potassium dihydrogen orthophosphate* and 4.68 g of *octane sulphonic acid* in 1000 ml of *water* and adjusted to pH 3.0 with *glacial acetic acid.*

Solvent mixture. 45 volumes of buffer solution and 55 volumes of *methanol.*

Test solution. Measure a volume of ophthalmic solution containing 20 mg of Timolol, add 15 ml of solvent mixture and dissolve with the aid of ultrasound for 10 minutes, allow to cool to room temperature, dilute to produce 25.0 ml with solvent mixture. Dilute 5.0 ml to 100.0 ml with solvent mixture.

Reference solution. A 0.001367 per cent w/v solution *timolol maleate IPRS* in solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- sample temperature: 5°,
- mobile phase: a mixture of 450 volumes of buffer solution, 530 volumes of *methanol* and 20 volumes of *tetrahydrofuran*,
- flow rate: 1 ml per minute,

– spectrophotometer set at 295 nm,
– injection volume: 20 µl.

Name	Relative retention time	Correction factor
Isotimolol	0.72	1.03
Timolol phenol	0.56	0.57
Timolol	1.0	---

The retention time of timolol is about 11.7 minutes, Isotimolol is about 8.37 minutes and timolol phenol is about 6.52 minutes.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injection is not more than 10.0 per cent.

Inject the test solution. The area of the peaks due to Isotimolol, timolol phenol is not more than 1.0 per cent and the area of secondary peak is not more than 1.0 per cent and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalisation. Ignore the peak due to maleic acid at relative retention time of about 0.22.

For Latanoprost —

Solvent mixture. 40 volumes of *water* and 60 volumes of *isopropyl alcohol.*

Test solution. Measure a volume of ophthalmic solution containing 0.2 mg of Latanoprost, add 4.0 ml of *isopropyl alcohol* and dissolve with the aid of ultrasound for 10 minutes, dilute 10.0 ml with the same solvent.

Reference solution (a). Dissolve 5 mg of *latanoprost IPRS* in 75 ml of solvent mixture, shake, dilute to 100.0 ml with the same solvent.

Reference solution (b). A 1.0 per cent w/v solution of *latanoprost acid impurity IPRS* in the solvent mixture. Dilute 0.2 ml to 100.0 ml with the solvent mixture.

Reference solution (c). Dilute to 1.0 ml of reference solution (a) and 2.5 ml of reference solution (b) with 50.0 ml of solvent mixture.

Reference solution (d). Dilute to 2.0 ml of reference solution (c) with 10.0 ml of solvent mixture.

Chromatographic system
– a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
– mobile phase: A. dissolve 2.0 g of *sodium dihydrogen orthophosphate* in 800 ml of *water*, add 200.0 ml of *acetonitrile*, adjusted to pH 2.5 with *orthophosphoric acid*,

B. *acetonitrile*,

– a gradient programme using the conditions given below,
– flow rate: 0.5 ml per minute,
– spectrophotometer set at 210 nm,
– injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	82	18
11	82	18
20	80	20
42	60	40
53	60	40
54	45	55
59	45	55
60	82	18
70	82	18

Name	Relative retention time	Correction factor
Latanoprost acid impurity	0.42	0.95
Latanoprost	1	---

The retention time of latanoprost is about 48 minutes.

Inject reference solution (d). The test is not valid unless the relative standard deviation for replicate injection is not more than 15.0 per cent.

Inject the test solution. The area of latanoprost acid impurity is not more than 1.0 per cent, calculated by area normalisation.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

For Timolol —

Solvent mixture. 90 volumes of *water* and 10 volumes of *acetonitrile.*

Test solution. Measure a volume of ophthalmic solution containing 50.0 mg of Timolol, add 70 ml of solvent mixture and dissolve with the aid of ultrasound for 10 minutes, allow to cool to room temperature, dilute to 100.0 ml with solvent mixture. Dilute 5.0 ml to 50.0 ml with solvent mixture.

Reference solution. A 0.068 per cent w/v solution of *timolol maleate IPRS* in solvent mixture. Dilute 5.0 ml to 50.0 ml with solvent mixture.

Chromatographic system
– a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
– sample temperature: 5°,
– mobile phase: a mixture of 650 volumes of a buffer solution prepared by dissolving 11.1 g of *monobasic sodium phosphate* in 1000 ml of *water* and adjusted to pH 2.8 with *orthophosphoric acid* and 350 volumes of *methanol*,

- flow rate: 1.2 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 10 µl.

Inject the reference solution. Retention time of timolol peak is at about 4.0 minutes for timolol, the test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{24}N_4O_3S$.

For Latanoprost —

Solvent mixture. 500 volumes of *water*, 500 volumes of *acetonitrile* and 60 volumes of *isopropyl alcohol*.

Test solution. Measure a volume of Ophthalmic Solution containing 0.25 mg of Latanoprost, add 10 ml of solvent mixture and dissolve with the aid of ultrasound for 10 minutes, allow to cool to room temperature, dilute with solvent mixture to 25.0 ml, mix and filter.

Reference solution. A 0.01 per cent w/v solution of *latanoprost IPRS* in solvent mixture. Dilute to 5.0 ml to 50.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 5.0 cm x 4.6 mm, packed with octylsilane bonded to porous silica (3 µm),
- sample temperature: 5°,
- mobile phase: a mixture of 550 volumes of a buffer solution prepared by dissolving 1.882 g of *hexane sulphonic acid sodium salt* in 1000 ml of *water* and adjusted to pH 3.0 with *dilute orthophosphoric acid* and 450 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. Retention time of about 3.2 minutes for latanoprost, the test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

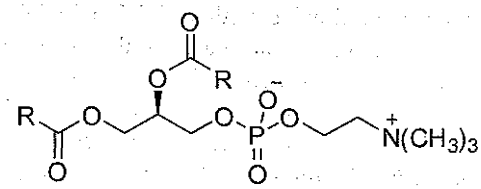
Inject the reference solution and the test solution.

Calculate the content of $C_{26}H_{40}O_5$.

Storage. Store protected from light and moisture. The container should be sterile, tamper evident and sealed so as to exclude microorganism.

Labelling. The label states the strength in terms of the equivalent amount of timolol and latanoprost in the labelled amount of ophthalmic solution.

Lecithin



Lecithin is a complex mixture of acetone-insoluble phosphatides, which consist chiefly of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol, combined with various amounts of other substances such as triglycerides, fatty acids and carbohydrates, as separated from the crude vegetable oil source.

Lecithin contains not less than 50.0 per cent of stated amount of acetone-insoluble matter.

Category. Pharmaceutical aid (excipient).

Identification

A. Transfer 1.0 g of Lecithin to a Kjeldahl flask, add 5.0 g of *potassium sulphate*, 0.5 g of *cupric sulpahte* and 20 ml of *sulphuric acid*. Incline the flask to a 45 degree angle, heat gently until the effervescence almost ceases and raise the temperature to boiling. After the contents become a blue, transparent solution, heat for 1 to 2 hours, cool and add an equal volume of *water*. To 5 ml of the solution, add 10 ml of 20 per cent w/v solution of *ammonium molybdate* and heat; a yellow precipitate is produced.

B. Determine by paper chromatography (2.4.15), coating the plate with cellulose.

Mobile phase. A mixture of 40 volumes of *n-butanol*, 20 volumes of *water* and 10 volumes of *acetic acid*.

Test solution. Dissolve 0.5 g of substance under examination in 5.0 ml of 50 per cent w/v solution of *hydrochloric acid*, heat in a water-bath for 2 hours, filter.

Reference solution. A 0.5 per cent w/v solution of *choline chloride IPRS*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of air, spray with *dragendorff's reagent* and examine in day light. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with the reference solution.

Tests

Acid value. Not more than 36 mg of potassium hydroxide, determined by the following method (2.3.23). Weigh 2.0 g of substance under examination in a 250-ml conical flask and dissolve it in 50 ml of *petroleum ether (40° to 60°)*, add 50 ml of *ethanol (95 per cent)*, previously neutralized to *phenolphthalein solution* with *0.1 M sodium hydroxide*, mix. Add *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* to a pink end point that persists for 5 seconds. Calculate the number of mg of *potassium hydroxide* required to neutralize the free acids in 1.0 g of lecithin.

Peroxide value. Not more than 10.

Weigh 5.0 g of substance under examination into a 250 ml Erlenmeyer flask with a ground-glass stopper; add 35 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *acetic acid*, mix. Completely dissolve the substance while shaking gently. The solution becomes transparent. Completely replace the air in the flask with nitrogen. While purging with nitrogen, add 1 ml of 16.5 per cent w/v solution of *potassium iodide*, then stop the flow of the nitrogen and immediately place a stopper in the flask. Shake for 1 minute and allow to stand in a dark place for 5 minutes, add 75 ml of *water*, replace the stopper again, shake vigorously. Titrate with *0.01 M sodium thiosulphate*, adding starch as the endpoint is approached and continue the titration until the blue colour of starch has just disappeared. Carry out a blank titration.

Calculate the content of peroxide value.

Water (2.3.43). Not more than 1.5 per cent, determined on 1 g.

Hexane-insoluble matter. Not more than 0.3 per cent. Weigh 10.0 g into a 250-ml conical flask, add 100 ml of *hexane* and shake until solution is apparently complete or until no more of any residue seems to be dissolving. Pass through a coarse-porosity filtering funnel that previously has been heated at 105° for 1 hour, cooled and weighed, wash the flask with two 25 ml portions of *hexane* and pour both washings through the funnel. Dry the funnel at 105° for 1 hour. Cool to room temperature and determine the gain in weight: not more than 0.3 per cent is found.

Lead (2.3.15). Not more than 0.001 per cent.

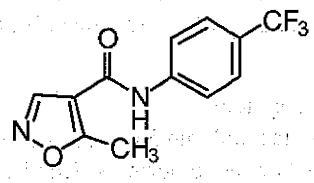
Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Acetone-insoluble matter. Weigh 2.0 g of substance under examination to a 40 ml centrifuge tube that previously has been tared along with a stirring rod, cool, Add 15.0 ml of *acetone*, warm carefully in a water-bath to melt the substance without evaporating the *acetone*, but with stirring to aid complete dissolution and place in an ice-water-bath for 5 minutes. Add *acetone* that previously has been chilled to 0° to 5° to the 40 ml mark on the tube, stirring during the addition. Cool in an ice-water-bath for 15 minutes, stir, remove the rod, clarify by centrifuging at about 2000 rpm for 5 minutes and decant. Break up the residue with the stirring rod and refill the centrifuge tube to the 40 ml mark with chilled *acetone*, while stirring. Cool in an ice-water-bath for 15 minutes, stir, remove the rod, centrifuge and decant. Break up the residue with the stirring rod. Place the tube in a horizontal position until most of the acetone has evaporated, mix again and heat the tube containing the acetone-insoluble residue and the stirring rod at 105° to constant weight. Determine the weight of the residue and calculate the percentage of acetone-insoluble matter.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states that it is flammable.

Leflunomide



$C_{12}H_9F_3N_2O_2$ Mol. Wt. 270.2

Leflunomide is 5-Methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide.

Leflunomide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_9F_3N_2O_2$, calculated on the dried basis.

Category. Antirheumatoid arthritis.

Description. A white to off-white powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *leflunomide IPRS* or with the reference spectrum of leflunomide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Impurity A (4-(trifluoromethyl)aniline). Not more than 0.02 per cent.

Determine by liquid chromatography (2.4.14) as described under Assay with the following modifications.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of a mixture of 10 volumes of *acetonitrile* and 90 volumes of the mobile phase.

Reference solution. A 0.00005 per cent w/v solution of *leflunomide impurity A IPRS* in a mixture of 10 volumes of *acetonitrile* and 90 volumes of the mobile phase.

Inject the reference solution and the test solution.

Calculate the content of leflunomide impurity A.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay.

Inject reference solution (a). The test is not valid unless the resolution between the principal peak and leflunomide impurity C is not less than 1.0.

Name	Relative retention time
5-Methylisoxazole-carboxylic acid	0.05
Leflunomide Impurity B[1]	0.22
N-(2'-Trifluoromethylphenyl)-5-methylisoxazole-4-Carboxamide	0.29
2-Cyano-acetic acid-(4'-trifluoromethyl)-anilide	0.36
Leflunomide impurity C[2]	0.94
Leflunomide	1.0

[1]2-cyano-3-hydroxy-N-(4'-trifluoromethylphenyl)-crotene amide,

[2](N-(3'-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide).

Inject the test solution. Run the chromatogram twice the retention time of the principal peak. The area of any peak corresponding to leflunomide impurity B is not more than 0.3 per cent. The area of any other secondary peak is not more than 0.1 per cent. The sum of areas of other secondary peaks is not more than 0.4 per cent, calculated by area normalization.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum over *phosphorus pentoxide* at 60° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Dissolve 50 mg of the substance under examination in *acetonitrile* and dilute to 100.0 ml with mobile phase.

Reference solution (a). A solution containing 0.05 per cent w/v of *leflunomide IPRS*, 0.015 per cent w/v of *leflunomide impurity B IPRS* and 0.005 per cent w/v of *leflunomide impurity C IPRS* in the mobile phase (dissolve in *acetonitrile* and dilute with the mobile phase).

Reference solution (b). A 0.05 per cent w/v solution of *leflunomide IPRS* in the mobile phase (dissolve in *acetonitrile* and dilute with mobile phase).

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *acetonitrile*, 1 volume of *triethylamine* and 130 volumes of *water*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Name	Relative retention time
Leflunomide impurity B[1]	0.2
Leflunomide impurity C[2]	0.9
Leflunomide	1.0

[1]2-Cyano-3-hydroxy-N-(4'-trifluoromethylphenyl)-crotone amide,

[2]N-(3'-Trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide.

Inject reference solution (a). The test is not valid unless the resolution between the leflunomide and leflunomide impurity C is not less than 1.0.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{12}H_9F_3N_2O_2$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Leflunomide Tablets

Leflunomide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of leflunomide, $C_{12}H_9F_3N_2O_2$.

Usual strengths. 10 mg; 20 mg and 100 mg.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum similar to that of *leflunomide IPRS*.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 1000 ml of *water (for tablets containing 10 mg or 20 mg);* 1000 ml of *water* containing 0.6 per cent of *polyoxyethylene lauryl ether (for tablets containing 100 mg)*,

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution immediately, suitably diluted with the dissolution medium, if necessary, at the maximum at about 262 nm (2.4.7). Calculate the content of $C_{12}H_9F_3N_2O_2$ in the medium from the absorbance obtained from a solution of known concentration of *leflunomide IPRS* prepared by dissolving in minimum quantity of *methanol* and diluted with the dissolution medium.

Q. Not less than 70 per cent of the stated amount of $C_{12}H_9F_3N_2O_2$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 100 mg of Leflunomide in 20 ml of *acetonitrile* and dilute to 100.0 ml with the mobile phase, filter.

Reference solution. A 0.1 per cent w/v solution of *leflunomide IPRS* in the mobile phase (dissolve in minimum quantity of *acetonitrile* and dilute with the mobile phase).

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *acetonitrile*, 1 volume of *triethylamine* and 130 volumes of *water*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

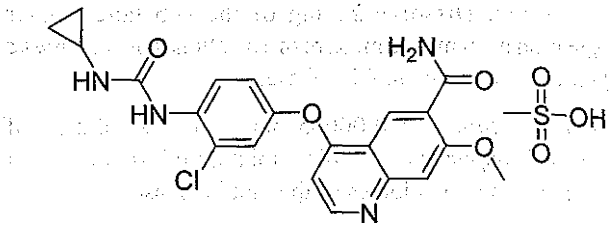
Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor for the principal peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{12}H_9F_3N_2O_2$ in the tablets.

Storage. Store protected from light and moisture.

Lenvatinib Mesylate



$C_{21}H_{19}ClN_4O_4CH_4O_3S$ Mol. Wt. 523.0

Lenvatinib Mesylate is 4-[3-chloro-4-(N'-cyclopropylureido) phenoxy]-7-methoxyquinoline-6-carboxamide methane sulphonate.

Lenvatinib Mesylate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{19}ClN_4O_4.CH_4O_3S$, calculated on anhydrous basis.

CAUTION — Lenvatinib Mesylate is cytotoxic, extra care required to prevent inhaling particles and exposing the skin to it.

Category. Anticancer.

Description. A white to pale reddish yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lenvatinib mesylate IPRS* or with the reference spectrum of lenvatinib mesylate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in chromatogram obtained with the reference solution.

Tests

Methane sulphonic acid content. 17.5 per cent to 19.5 per cent, calculated on anhydrous basis.

Dissolve 0.4 g in 50 ml of a mixture of 2 volumes of *dimethylsulphoxide*, 2 volumes of *methanol* and 1 volume of *water*. Titrate with *0.1 M sodium hydroxide*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.00961 g of *methane sulphonic acid*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture (a). Equal volumes of *methanol* and *water*.

Solvent mixture (b). 70 volumes of *methanol* and 30 volumes of *water*.

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 25 mg of substance under examination in 40 ml of *methanol* and dilute to 100.0 ml with solvent mixture (a).

Reference solution (a). Dissolve 25 mg of *lenvatinib mesylate IPRS* in 40 ml of *methanol* and dilute to 100.0 ml with solvent mixture (a). Dilute 1.0 ml of the solution to 25.0 ml with solvent mixture (b). Further, dilute 5.0 ml of the solution to 100.0 ml with solvent mixture (b).

Reference solution (b). Dilute 3.0 ml of reference solution (a) to 10.0 ml with solvent mixture (b).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as X-Bridge Shield RP-18),
- sample temperature: 10°,
- mobile phase: A. a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.25 with *dilute orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 15 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
15	70	30
20	70	30
35	60	40
45	60	40
55	30	70
57	90	10
65	90	10

Name	Relative retention time	Correction factor
Descyclopropyl lenvatinib[1]	0.65	0.78
Methyl analogue of lenvatinib[2]	0.79	0.79
Lenvatinib carboxylic acid[3]	0.87	0.98
Lenvatinib (Retention time: about 17 minutes)		1.00
Carbamate derivative of APQC[4]	1.90	1.19
Carbamoyl derivative of lenvatinib[5]	2.18	0.99
Nitrile analogue of lenvatinib[6]	2.57	1.05

[1]4-[4-(carbamoylamino)-3-chlorophenoxy]-7-methoxyquinoline-6-carboxamide,

[2]4-(3-chloro-4-[(methylcarbamoyl)amino]phenoxy)-7-methoxyquinoline-6-carboxamide,

[3]4-(3-chloro-4-[(cyclopropylcarbamoyl)amino]phenoxy)-7- methoxyquinoline-6- carboxylic acid,

[4]phenyl(4-[(6-carbamoyl-7-methoxyquinolin-4-yl)oxy]-2-chlorophenyl)carbamate,

[5]4-(3-chloro-4-[(cyclopropylcarbamoyl)amino]phenoxy)-N-(cyclopropylcarbamoyl)-7- methoxyquinoline-6- carboxamide,

[6]1-(2-chloro-4-[(6-cyano-7- methoxyquinolin-4- yl)oxy]phenyl)-3-cyclopropylurea.

Inject reference solution (a) and (b). The test is not valid unless the column efficiency is not less than 50000 theoretical plates, the tailing factor is not more than 1.5, the relative standard deviation for replicate injections is not more than 10.0 per cent in the chromatogram obtained with reference solution (a) and the signal-to-noise ratio is not less than 10 in the chromatogram obtained with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to descyclopropyl lenvatinib, methyl analogue of lenvatinib, lenvatinib carboxylic acid, carbamate derivative of APQC, carbamoyl derivative of lenvatinib and nitrile analogue of lenvatinib, each of, is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any other secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 7.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture (a). Equal volumes of *methanol* and *water*.

Solvent mixture (b). 70 volumes of *methanol* and 30 volumes of *water*.

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 50 mg of the substance under examination in 40 ml of *methanol* and dilute to 100.0 ml with solvent mixture (a). Dilute 5.0 ml of the solution to 100.0 ml with solvent mixture (b).

Reference solution. Dissolve 50 mg of *lenvatinib mesylate IPRS* in 40 ml of *methanol* and dilute to 100.0 ml with solvent mixture (a). Dilute 5.0 ml of the solution to 100.0 ml with solvent mixture (b).

Solvent mixture (c). 70 volumes of *methanol* and 30 volumes of *water.*

Test solution. Disperse a suitable quantity of intact capsules containing 40 mg of the Lenvatinib in 100 ml of solvent mixture (a), with the aid of magnetic stirrer for 20 minutes. Add 50 ml of solvent mixture (b), sonicate for 10 minutes with intermittent shaking and dilute to 200.0 ml with solvent mixture (b). Dilute 2.0 ml of the solution to 10.0 ml with solvent mixture (c).

Reference solution. Dissolve 50 mg of *lenvatinib mesylate IPRS* in 40 ml of *methanol* and dilute to 100.0 ml with solvent mixture (b). Dilute 1.0 ml of the solution to 10.0 ml with solvent mixture (c).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as XBridge C 18),
- column temperature: 40°,
- sample temperature: 10°,
- mobile phase: A. a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water,* adjusted to pH 3.35 with *dilute orthophosphoric acid,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
8	75	25
10	35	65
15	35	65
17	75	25
25	75	25

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

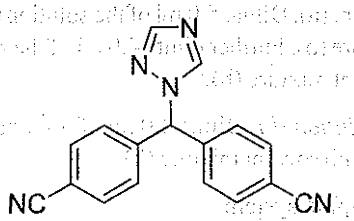
Calculate the content of $C_{21}H_{19}ClN_4O_4$ in the capsules.

1 mg of the lenvatinib mesylate, $C_{21}H_{19}ClN_4O_4$, CH_4O_3S is equivalent to 0.816 mg of lenvatinib, $C_{21}H_{19}ClN_4O_4$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of lenvatinib.

Letrozole



$C_{17}H_{11}N_5$ Mol Wt. 285.3

Letrozole is benzonitrile, 4,4'-(1H-1,2,4-triazol-1-ylmethylene) bis; 4,4'-(1H-1,2,4-Triazol-1-ylmethylene) dibenzonitrile.

Letrozole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{11}N_5$, calculated on the anhydrous basis.

Category. Aromatase inhibitor; treatment of breast cancer.

Description. A white or almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *letrozole IPRS* or with the reference spectrum of letrozole.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *water* and 30 volumes of *acetonitrile.*

Test solution. Dissolve 25 mg of the substance under examination in the 250-ml volumetric flask add 75 ml of *acetonitrile,* mix and dilute with *water* to volume.

Reference solution (a). A 0.0002 per cent w/v solution of *letrozole related compound A IPRS* and 0.001 per cent w/v solution of *letrozole IPRS* in the solvent mixture.

Reference solution (b). A 0.0001 per cent w/v solution of *letrozole IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *water,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
25	30	70
25.1	70	30
30	70	30

Name	Relative retention time
Letrozole related compound A[1]	0.67
Letrozole	1.0
4,4',4"-Methanetriyltribenzonitrile	2.4
Individual impurity	---

[1]4,4'-(1*H*-1,3,4-triazol-1-ylmethylene) dibenzonitrile.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to letrozole related compound A and letrozole is not less than 2.0.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 10.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with test solution, the area of impurity due to letrozole related compound (A) is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of impurity due to 4,4',4"-Methanetriyltribenzonitrile is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Test solution. Dissolve 10 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.001 per cent w/v solution of *letrozole IPRS* in the solvent mixture.

Inject reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{11}N_5$.

Storage. Store protected from light and moisture.

Letrozole Tablets

Letrozole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of letrozole, $C_{17}H_{11}N_5$.

Usual strength. 2.5 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 70 volumes of *water* and 30 volumes of *acetonitrile*.

Mobile phase. A mixture of 90 volumes of *ethyl acetate* 10 volumes of *methanol*.

Test solution. Disperse a quantity of the powdered tablets containing 2 mg of letrozole in 1 ml of *methanol*, ultrasound for 10 minutes and centrifuge.

Reference solution. A 0.2 per cent w/v solution of *letrozole IPRS* in *methanol*.

Apply to the plate 5 µl of reference solution and test solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spots obtained in the chromatogram obtained with the test solution correspond to that in the chromatogram obtained with the reference solution .

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.1 M hydrochloric acid*.
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Weigh accurately a suitable quantity of *letrozole IPRS,* dissolve in minimum quantity of *acetonitrile* and dilute with the dissolution medium to obtain a solution having the similar concentration as that of the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 480 volumes of *acetonitrile* and 520 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{11}N_5$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{11}N_5$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *water* and 30 volumes of *acetonitrile.*

Test solution. Weigh a quantity of powders tablets containing 10 mg of Letrozole, disperse in 20 ml of solvent mixture with the aid of ultrasound for 10 minutes, cool dilute to 100.0 ml with solvent mixture. Mix well and centrifuge, use the supernatant liquid.

Reference solution (a). A 0.0002 per cent w/v solution of *letrozole related compound A IPRS* and 0.001 per cent w/v solution of *letrozole IPRS* in the solvent mixture.

Reference solution (b). A 0.0001 per cent w/v solution of *letrozole IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
25	30	70
25.1	70	30
30	70	30

Name	Relative retention time
Letrozole related compound A[1]	0.67
Letrozole	1.0
4,4',4"-Methanetriyltribenzonitrile	2.4
Individual impurity	—
Total impurity	—

[1]4,4'-(1*H*-1,3,4-triazol-1-ylmethylene) dibenzonitrile, this is process impurity.

(*NOTE — Letrozole related compound A and 4,4',4"-Methanetriyltribenzonitrile are process impurities and are controlled in the drug substance monograph*)

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to letrozole related compound A and letrozole is not less than 2.0.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 10.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 0.3 times the area of the peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as given under Assay using the following test solution.

Test solution. Transfer one tablet to a 25.0 ml volumetric flask, add about 1 ml of *water* and swirl to disperse the tablet, add 7.5 ml of *acetonitrile* and ultrasound for about 20 minutes. Cool and dilute with *water* to volume. Shake, mix well and filter, rejecting the first few ml of filtrate. Dilute further suitably with mobile phase to produce a solution containing 0.001 per cent w/v of letrozole.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{11}N_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *water* and 30 volumes of *acetonitrile.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Letrozole in 100-ml

volumetric flask, shake with 10 ml *water* for 5.0 minutes, mix add 50.0 ml *acetonitrile,* shake for 30.0 minutes, dilute to produce 100.0 ml with *water*. Dilute 5.0 ml to 100.0 ml in the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *letrozole IPRS* in *Solvent mixture*. Dilute to 5.0 ml to 100.0 ml in *mobile phase.*

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 48 volumes of *acetonitrile and* 52 volumes of *water,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

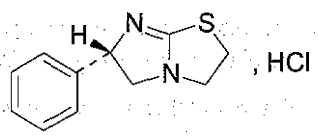
Inject the reference solution. The tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{11}N_5$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Levamisole Hydrochloride



$C_{11}H_{12}N_2S,HCl$ Mol. Wt. 240.8

Levamisole Hydrochloride is (*S*)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole hydrochloride.

Levamisole Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{11}H_{12}N_2S,HCl$, calculated on the dried basis.

Category. Anthelmintic.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B and D may be omitted if tests A, C and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levamisole hydrochloride IPRS* or with the reference spectrum of levamisole hydrochloride.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Complies with the test for Specific optical rotation.

D. Dissolve 0.5 g in 20 ml of *water* and add 6 ml of *1 M sodium hydroxide.* Extract with 20 ml of *dichloromethane*, wash the lower layer with two quantities, each of 10 ml, of *water*, dry over *anhydrous sodium sulphate*, filter and evaporate the solvent at a temperature not exceeding 40° under reduced pressure. The residue melts at 58° to 61° (2.4.21).

E. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 3.0 to 4.5, determined in a 5.0 per cent w/v solution.

Specific optical rotation (2.4.22). –128° to –121°, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use, protect from light and keep below 25°.

Test solution. Dissolve 0.1 g of the substance under examination in *methanol,* add 1.0 ml of *ammonia* and dilute to 10.0 ml with *methanol.*

Reference solution. Dissolve 10 mg of the *levamisole hydrochloride IPRS* in *methanol,* add 1.0 ml of *ammonia* and dilute to 100.0 ml with *methanol.* Further dilute 5.0 ml of the solution to 25.0 ml with *methanol.*

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with base- deactivated octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. dissolve 0.5 g of *ammonium dihydrogen phosphate* in 90 ml of *water*, adjusted to pH 6.5 with a 4.0 per cent w/v solution of *sodium hydroxide* and dilute to 100 ml with *water*,

  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
8	30	70
10	30	70
12	90	10
17	90	10

Name	Relative retention time	Correction factor
Levamisole impurity A[1]	0.9	2.0
Levamisole (Retention time: about 3 minutes)	1.0	—
Levamisole impurity B[2]	1.4	1.7
Levamisole impurity C[3]	1.5	2.9
Levamisole impurity D[4]	1.6	1.3
Levamisole impurity E[5]	2.0	2.7

[1]3-[(2*RS*)-2-amino-2-phenylethyl]thiazolidin-2-one,

[2]3-[(*E*)-2-phenylethenyl]thiazolidin-2-imine,

[3](4*RS*)-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one,

[4]6-phenyl-2,3-dihydroimidazo[2,1-*b*]thiazole,

[5]1,1'-[(disulphane-1,2-diyl)bis(ethylene)]bis[(4*RS*)-4-phenylimidazolidin-2-one].

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is not more than 3.5.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of peaks corresponding to levamisole impurities A, B, C, D and E is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh 0.2 g, dissolve in 30 ml of *ethanol (95 per cent)*, add 5 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (2.4.25). Record the volume added between the two inflections. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02408 g of $C_{11}H_{12}N_2S,HCl$.

Storage. Store protected from light and moisture.

Levamisole Tablets

Levamisole Hydrochloride Tablets

Levamisole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levamisole, $C_{11}H_{12}N_2S$.

Usual strengths. The equivalent of 50 mg, 150 mg of levamisole.

Identification

In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 214 nm (2.4.7). Calculate the content of $C_{11}H_{12}N_2S$, in the medium from the absorbance obtained by repeating the determination using a solution of *levamisole IPRS* in the same medium.

Q. Not less than 80 per cent of the stated amount of $C_{11}H_{12}N_2S$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

Mobile phase. A mixture of 60 volumes of *toluene*, 40 volumes of *acetone* and 1 volume of *strong ammonia solution.*

Test solution (a). Shake a quantity of the powdered tablets containing 100 mg of levamisole with 5 ml of *methanol* for 2 minutes and filter.

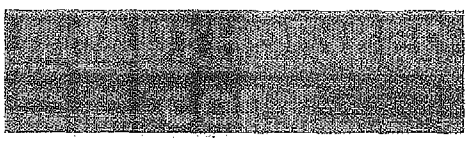
Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *methanol.*

Reference solution (a). A 0.24 per cent w/v solution of *levamisole hydrochloride IPRS* in *methanol.*

Referece solution (b). Dilute 1 ml of reference solution (a) to 20 ml with *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate at 105° for 15 minutes and examine under ultraviolet light at 254 nm as well as after exposure to iodine vapour. Any secondary spot the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.



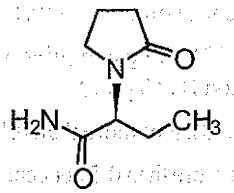
Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 300 mg of levamisole, add 30 ml of *water* and shake for 10 minutes. Filter, wash the filter with 20 ml of *water* and add the washings to the filtrate. To the combined filtrate add *dilute ammonia solution* to make it alkaline and extract with three quantities each of 25 ml, 15 ml and 15 ml, of *chloroform*, filter through cotton wool covered with a layer of *anhydrous sodium sulphate*. Combine the chloroform extracts and evaporate to dryness. Dissolve the residue in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02043 g of $C_{11}H_{12}N_2S$.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of levamisole.

Levetiracetam



$C_8H_{14}N_2O_2$ Mol. Wt. 170.2

Levetiracetam is 1-Pyrrolidineacetamide, α-ethyl-2-oxo-, (αS).

Levetiracetam contains not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_{14}N_2O_2$, calculated on the anhydrous and solvent-free basis.

Category. Antiepileptic.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levetiracetam IPRS* or with the reference spectrum of levetiracetam.

B. In the test for enantiomeric purity, the principal peak in the chromatogram obtained with reference solution (c) corresponds to the levetiracetam s-enantiomer peak in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 125 mg of the substance under examination in mobile phase (a) and dilute to 25.0 ml with mobile phase (a).

Reference solution. A 0.0005 per cent w/v solution of *levetiracetam IPRS* in mobile phase (a).

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a mixture of 95 volumes of a buffer solution prepared by dissolving 2.7 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 5.5 with 2 per cent w/v solution of *potassium hydroxide* and 5 volumes of *acetonitrile*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
3	100	0
20	71	29
25	100	0
28	100	0

Name	Relative retention time	Correction factor
Levetiracetam impurity C[1]	0.37	
Levetiracetam acid[2]	0.62	0.83
Levetiracetam	1.0	
Levetiracetam impurity A[3]	1.25	2.85

[1]Pyridin-2-ol (Not included in the total impurities limit).

[2](*S*)-2-(2-0xopyrrolidin-1-yl) butanoic acid.

[3]*(S)-N-(1*-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to levetiracetam impurity C is not more than 0.25 times the area of the principal peak in a chromatogram obtain with the reference solution (0.025 per cent), the area of any peak corresponding to levetiracetam acid is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent), the area of any peak corresponding to levetiracetam impurity A and any other secondary peak is not more than 0.5 times the area of

the principal peak in the chromatogram obtain with the reference solution (0.05 per cent) and the sum of areas of all the secondary peaks including impurity B obtained from the test for Levetiracetum impurity B is not more than 4 times the area of the principal peak in the chromatogram obtain with the reference solution (0.4 per cent). Ignore any peak with a relative retention time of 0.19 or less.

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.005 per cent w/v solution of *levetiracetam IPRS* in the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *levetiracetam racemic mixture IPRS* in the mobile phase.

Reference solution (c). Dilute 5.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, amylase tris-3,5-dimethylphenylcarbamate (AD-H) bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *n-hexane* and 20 volumes of *dehydrated alcohol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

The relative retention time with respect to levetiracetam *S*-enantiomer for levetiracetam *R*-enantiomer is about 0.55.

Inject reference solution (b).The test is not valid unless the resolution between the peak due to levetiracetam *R*-enantiomer and levetiracetam *S*-enantiomer is not less than 4.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to levetiracetam *R*-enantiomer (impurity D) is not more than 1.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent).

Levetiracetam impurity B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in the mobile phase and dilute to 100.0 ml with mobile phase.

Reference solution (a). A 0.2 per cent w/v solution of *levetiracetam impurity B IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of a buffer solution prepared by dissolving 1.22 g of *sodium1-decanesulfonate* in 1000 ml of *water*, add 1.3 ml of *orthophosphoric acid*, adjusted to pH 3.0 with 20 per cent w/v solution of *potassium hydroxide* and 15 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 50 µl, for system suitability10 µl.

The retention time for levetiracetam impurity B is about 9 minutes.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 3.0. and the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to levetiracetam impurity B is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Test solution. Dissolve 0.1 g of the substance under examination in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 1.0 ml of the solution to 10.0 ml with mobile phase A.

Reference solution. A 0.01 per cent w/v solution of *levetiracetam IPRS* in mobile phase A.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{14}N_2O_2$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Levetiracetam Oral Solution

Levetiracetam Oral Solution is a solution of levetiracetam in a suitable aqueous vehicle.

Levetiracetam Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levetiracetam $C_8H_{14}N_2O_2$.

Usual strength. 500 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.8 to 6.3.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 5 volumes of *acetonitrile* and 95 volumes of *orthophosphoric acid* solution prepared by diluting 2.0 ml of *orthophosphoric acid* to 1000.0 ml with *water*.

Test solution. Dilute a volume of the oral solution containing 200 mg of levetiracetam in 60 ml of mobile phase A, mix with the aid of ultrasound for 5 min, allow the solution to cool and dilute it to 100.0 ml with mobile phase A.

Reference solution (a). Dissolve 20 mg of *levetiracetam IPRS* in 10 ml of *0.1M potassium hydroxide* allow to stand for 15 minute to react and then neutralize by adding of 10 ml of *0.1M hydrochloric acid.* Add 10 mg of *levetiracetam impurity A IPRS*, mix with the aid of ultrasound and dilute it to 100.0 ml with the solvent mixture.

Reference solution (b). A 0.0003 per cent w/v solution of *levetiracetam IPRS* in the mobile phase A.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: A. a solution of *orthophosphoric acid* prepared by diluting 2.0 ml of *orthophosphoric acid* in 1000 ml with *water.*

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
7	95	5
20	90	10
30	75	25
35	50	50
40	50	50
41	100	0
50	100	0

Name	Relative retention time	Correction factor
Levetiracetam	1.00	—
Lvetiracetam impurity A[1]	1.38	—
Levetiracetam acid[2]	1.46	1.08

[1](S)-N-(1-Amino-1-oxobutan-2yl)-4-chlorobutanamide (This is a process impurity and include for peak identification only),

[2](S)-2-(2-Oxopyrrolodin-1-yl) butanoic acid.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to levetiracetam impurity A and levetiracetam acid is not less than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to levetiracetam acid is not more than twice the area of principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent) and the area of any other secondary peak is not more than 0.66 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1per cent). The sum of the areas of all the secondary peaks is not more than 6.66 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the oral solution containing 200 mg of levetiracetam, in 120 ml of mobile phase A, mix with the aid of ultrasound for 5.0 minutes. Allow the solution to cool and dilute it to 200.0 ml with the mobile phase A.

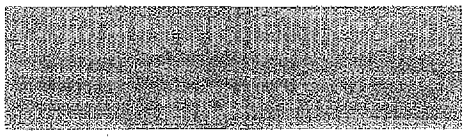
Refrence solution. A 0.1 per cent w/v solution of *levetiracetam IPRS* in the mobile phase A.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a solution of *orthophosphoric acid* prepared by diluting 1.0 ml of *orhophophoric acid* in 1000 ml with *water*,

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	92	8
6	92	8
7	40	60
10	40	60
11	92	8
15	92	8



Inject the reference solution. The test is not valid unless the tailing factor for the principal peak is not more than 2.0 per cent and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{14}N_2O_2$ in the oral solution.

Storage. Store protected from light, at a temperature not exceeding 30°.

Levetiracetam Prolonged-release Tablets

Levetiracetam Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Levetiracetam Prolonged-release Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levetiracetam, $C_8H_{14}N_2O_2$.

Usual strengths. 500 mg; 750 mg; 1000 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 5 ml of *acetonitrile* and 95 ml of solution of *orthophosphoric acid* prepared by diluting 2ml of *orthophosphoric acid* with *water* to 1000 ml.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.25 g of levetiracetam in 80 ml of water, mix with the aid of ultrasound for 10 minutes and dilute it with *water* to 100.0 ml, filter.

Reference solution (a). Dissolve 30 mg of *levetiracetam IPRS* in 10 ml of *0.1M potassium hydroxide* allow to stand the solution for 5 minutes to react and then neutralize by adding 10 ml of *0.1M hydrochloric acid* dilute to 100.0 ml with the solvent mixture. *[NOTE—This solution contains levetiracetam and levetiracetam acid.]*

Reference solution (b). A 0.00125 per cent w/v solution of *levetiracetam IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 1 g of *sodium hexanesulphonate monohydrate* in 1000 ml of a solution containing a mixture of 5 volumes of *acetonitrile* and 95 volumes of buffer solution prepared by dissolving 1.4 g of *disodium hydrogen phosphate, anyhydrous* in *water*, adjusted to pH 3.5 with *orthophosphoric acid,*
- flow rate: 2 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

Name	Relative retention time
Levetiracetam impurity B[1]	0.40
Levetiracetm	1.0
Levetiracetam acid[2]	1.3
Levetiracetam impurity A[3]	1.9

[1](S)-2-Aminobutanamide,

[2](S)-2-(2-Oxopyrrolidin-1-yl)butanoic acid,

[3](S)-N-(1-Amino-1-oxobutan-2-yl)-4-chlorobutanamide.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to levetiracetam acid impurity and levetiracetam is not less than 1.5 in the chromatogram obtained with reference solution (a) and the tailing factor is not more than 2.0 in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to levetiracetam acid is not more than 0.6 times the area of principal peak in the chromatogram obtained with the reference solution (b) (0.3 per cent) and the area of any other secondary peak is not more than the 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.1 per cent). The sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determined by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.4 g of levetirecetam in 5 ml of *tetrahydrofuran.* Stir for 30 minutes and allow to stand for 5 minutes. Mix with the aid of ultrasound. Add 75 ml of mobile phase, mix with the aid of ultrasound for 30 min, add 10 ml of *methanol* and finally dilute to 100.0 ml with the mobile phase if necessary, filter. Dilute 2.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. Dissolve 80 mg of *levetiracetam IPRS* in 60 ml of mobile phase, add 4 ml of *tetrahydrofuran*, mix with the aid of ultrasound and dilute it to 100.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of buffer solution prepared by dissolving 1.4 g of *anyhydrous dibasic sodium phosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *orthophosphoric acid.*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_8H_{14}N_2O_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Levetiracetam Tablets

Levetiracetam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levetiracetam, $C_8H_{14}N_2O_2$.

Usual strengths. 250 mg; 500 mg; 750 mg; 1000 mg.

Identification

A. Transfer a quantity of the finely powdered tablets containing 250 mg of Levetiracetam to a 50-ml volumetric flask, add 35 ml of the *acetone*, sonicate for 15 minutes and dilute to 50.0 ml with *acetone*, filter 10.0 ml solution through a membrane filter and evaporate the filtrate to dryness for crystallisation, scratching the sides of the vessel, on the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 0.1 per cent w/v solution of *levetiracetam IPRS* in *acetone*, treated in the same manner, beginning with "10 ml solution" or with the reference spectrum of levetiracetam.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), as described under assay with the following modifications.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution containing 0.04 per cent w/v of *levetiracetam IPRS* in the solvent mixture and sonicate, dilute further, if necessary.

Q. Not less than 80 per cent of the stated amount of $C_8H_{14}N_2O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 120 mg of Levetiracetam in the mobile phase, dilute to 100.0 ml with mobile phase and filter.

NOTE — Sonicate if necessary and centrifuge the solution before passing through a suitable filter.

Reference solution (a). A solution containing 0.00036 per cent w/v each of *levetiracetam IPRS* and *levetiracetam impurity B IPRS* in *mobile phase.*

Reference solution (b). A solution containing 0.00036 per cent w/v of *Levetiracetam IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 95 volumes of a buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* and 0.85 g of *sodium 1-heptanesulphonate* in 1000 ml of *water* and adjusted to pH 2.8 with *orthophosphoric acid* and 5 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Levetiracetam impurity B[1]	0.54	—
Levetiracetam	1.0	—
Levetiracetam impurity A[1,2]	1.7	—
Levetiracetam acid[3]	2.1	1.27

[1] (*S*)-2-aminobutanamide hydrochloride, this is process impurity,

[2](*S*)-*N*-(1-amino-1-oxobutan-2-yl)-4-chlorobutanamide,

[3](*S*)-2-(2-oxopyrrolidine-1-yl)butanoic acid.

Inject reference solution (a) and (b). The test is not valid unless the resolution between levetiracetam impurity B and

levetiracetam is not less than 2.0 obtained with reference solution (a), the tailing factor for levetiracetam peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 10.0 per cent obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to levetiracetam acid is not more than area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than 0.33 the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *acetonitrile* and 80 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Transfer a quantity of the powder containing about 40 mg of Levetiracetam in 100-ml volumetric flask and add about 80 ml of solvent mixture and disperse with aid the aid of ultrasound for about 10 minutes, cool and dilute to volume with solvent mixture and filter.

Reference solution. A solution containing 0.04 per cent w/v of *levetiracetam IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: a mixture of 92 volumes of a buffer solution prepared by dissolving 1.4 g of *monobasic potassium phosphate* and 0.6 g of *sodium 1-haptanesulphonate* in 1000 ml of *water* and adjusted to pH 2.8 with *ortho phosphoric acid* and 8 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

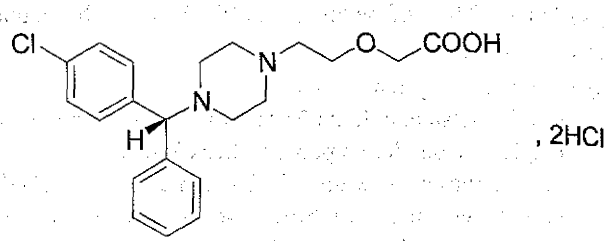
Inject the reference solution and the test solution.

Calculate the content of $C_8H_{14}N_2O_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Levocetirizine Hydrochloride

Levocetirizine Dihyrochloride



$C_{21}H_{25}N_2O_3Cl,2HCl$ Mol. Wt. 461.8

Levocetirizine Hydrochloride is (*R*)-2-[2-[4-[(4-chloro-phenyl) phenylmethyl]piperazin-1-yl]ethoxy]acetic acid dihydrochloride.

Levocetirizine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{25}N_2O_3Cl,2HCl$ calculated on the dried basis.

Category. Antihistaminic.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levocetirizine dihydrochloride IPRS* or with the reference spectrum of levocetirizine dihydrochloride.

B. When examined in the range 200 nm and 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 230 nm.

Tests

Specific optical rotation (2.4.22). + 10° to +14°, determined in a 1 per cent w/v solution in *carbon dioxide-free water* at 365 nm.

Heavy metals (2.3.13). Dissolve the residue obtained in the test for sulphated ash in 20 ml *water*. 12 ml of the solution complies with limit test for heavy metals, Method D (20 ppm).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 12.5 mg of the substance under examination in 1 ml of *ethanol (95 per cent)* and dilute to 25 ml with the mobile phase, filter.

Reference solution. Dissolve 12.5 mg of the *racemic cetirizine dihydrochloride IPRS* in 1 ml of *ethanol (95 per cent)* and dilute to 25 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with chiral Pak AD-H (5 µm),

- mobile phase: a mixture of 70 volumes of *n-hexane*, 15 volumes of *isopropyl alcohol*, 15 volumes of *ethanol (95 per cent)*, 0.2 volume of *trifluoro acetic acid* and 0.01 volume of *diethylamine*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution and the test solution.

The relative retention time of levocetirizine isomer is about 2 with respect to levocetirizine peak.

Calculate the chiral purity of levocetirizine dihydrochloride by area normalization method, the enantiomeric purity is not less than 98 per cent.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use the solutions within 16 hours.

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase, filter.

Reference solution (a). A solution containing 0.00002 per cent w/v, each of, *levocetirizine dihydrochloride IPRS*, *levocetirizine amide IPRS* and *chlorobenzhydryl piperazine IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.02 per cent w/v of *levocetirizine dihydrochloride IPRS* and 0.00002 per cent w/v, each of, *levocetirizine amide IPRS* and *chlorobenzhydryl piperazine IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with silica gel (5 µm),
- mobile phase: a mixture of 93 volumes of *acetonitrile*, 6.6 volumes of *water* and 0.4 volume of *1M sulphuric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Name	Relative retention time
Levocetirizine	1.0
Chlorobenzhydryl piperazine[1]	1.4
Levocetirizine amide[2]	2.1

[1](R)-1-[(4-chlorophenyl)phenylmethyl]piperazine,

[2](R)-2-(2-{4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy) acetamide.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to chlorobenzhydryl piperzine and levocetirizine is not less than 3.0, the tailing factor for levocetirizine peak is not more than 2.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections for levocetirizine is not more than 5.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of levocetirizine peak. The area of any peak corresponding to chlorobenzhydryl piperzine or levocetirizine amide is not more than twice the area of the peak due to chlorobenzhydryl piperzine or levocetirizine amide in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than the area of the peak due to levocetirizine in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak due to levocetirizine in the chromatogram obtained with reference solution (a) (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on 2 g.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

Assay. Dissolve 0.1 g in 70 ml of a mixture of 30 ml of *water* and 70 ml of *acetone*. Titrate with *0.1 M sodium hydroxide* upto the second point of inflection. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01539 g of $C_{21}H_{25}N_2O_3Cl,2HCl$.

Storage. Store protected from moisture.

Levocetirizine Tablets

Levocetirizine Dihydrochloride Tablets

Levocetirizine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levocetirizine hydrochloride, $C_{21}H_{25}N_2O_3Cl,2HCl$.

Usual strength. 5 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *phosphate buffer pH 6.8*,

Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.025 per cent w/v solution of *levocetirizine dihydrochloride IPRS* in mobile phase. Dilute 1 ml of the solution to 50 ml with dissolution medium.

Use chromatographic system as described under Assay.

Calculate the content of $C_{21}H_{25}N_2O_3Cl,2HCl$.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{25}N_2O_3Cl,2HCl$ in the medium.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 50 mg of Levocetirizine Dihydrochloride, in 20 ml of a mixture of 5.7 volumes of *1 M of sulphuric acid* and 94.3 volumes of *water*, with the aid of ultrasound with intermittent shaking. Add 150 ml of *acetonitrile*, place the flask in an ultrasonic bath for 10 minutes, cool and dilute to 250.0 ml with *acetonitrile*. Centrifuge the solution and use the supernatant liquid.

Reference solution (a). A 0.0002 per cent w/v solution of *levocetirizine dihydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.02 per cent w/v of *levocetirizine dihydrochloride IPRS* and 0.00002 per cent w/v of *chlorobenzhydryl piperazine IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with silica gel (5 µm),
- mobile phase: a mixture of 93 volumes of *acetonitrile*, 6.6 volumes of *water* and 0.4 volume of *1M sulphuric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Name	Relative retention time
Levocetirizine	1.0
Chlorobenzhydryl piperazine[1,*]	1.4
Levocetirizin amide[2,*]	2.1

*Process impurity controlled in drug substance and no need to report in drug product,

[1](*R*)-1-[(4-chlorophenyl)phenylmethyl]piperazine,

[2](*R*)-2-(2-{4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetamide.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to chlorobenzhydryl piperzine and levocetirizine is not less than 3.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections for levocetirizine is not more than 2.0 per cent and for chlorobenzhydryl piperzine is not more than 5.0 per cent.

Inject reference solution (a) and the test solution. Run the chromatogram 2.3 times the retention time of levocetirizine peak, the area of any secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using following modifications.

Test solution. Transfer 1 tablet in a suitable volumetric flask. Add about 20 ml of mobile phase to disperse with shaking. Further dilute with the mobile phase to obtain a final concentration of 0.0025 per cent w/v.

Reference solution. A 0.0025 per cent w/v solution of *levocetirizine dihydrochloride IPRS* in the mobile phase.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powdered tablet containing 25 mg of Levocetirizine Dihydrochloride, disperse in 100.0 ml of mobile phase and filter. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

Reference solution. A 0.005 per cent w/v solution of *levocetrizine dihydrochloride IPRS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *0.05 M potassium dihydrogen phosphate* and 40 volumes of *acetonitrile*, adjusted to pH 6.0 with 10 per cent w/v of *sodium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0. The column efficiency in not less than 1500 theoretical plates. The relative standard deviation for replicate injections is not more than 2.0 per cent.

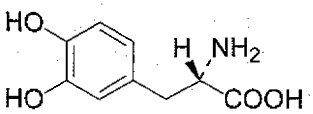
Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{25}N_2O_3Cl,2HCl$.

Storage. Store protected from light and moisture.

Levodopa

L-Dopa



$C_9H_{11}NO_4$ Mol. Wt. 197.2

Levodopa is 3-(3,4-dihydroxyphenyl)-L-alanine.

Levodopa contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_{11}NO_4$, calculated on the dried basis.

Category. Antiparkinsonian.

Description. A white or slightly cream, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levodopa IPRS* or with the reference spectrum of levodopa.

B. Dissolve about 2 mg in 2 ml of *water* and add 0.2 ml of *ferric chloride solution*; a green colour develops which changes to bluish violet on the addition of 0.1 g of *hexamine*.

C. Dissolve about 5 mg in a mixture of 5 ml of *1 M hydrochloric acid* and 5 ml of *water*. Add 0.1 ml of *sodium nitrite solution* containing 10 per cent w/v of *ammonium molybdate*; a yellow colour develops which changes to red on the addition of *10 M sodium hydroxide*.

D. Mix about 5 mg with 1 ml of *water*, 1 ml of *pyridine* and about 5 mg of *4-nitrobenzoyl chloride* and allow to stand for 3 minutes; a violet colour develops which changes to pale yellow on boiling. Add, while shaking, 0.2 ml of *sodium carbonate solution*; the violet colour reappears.

Tests

Appearance of solution. A 4.0 per cent w/v solution in *1 M hydrochloric acid* is not more intensely coloured than reference solution BYS6 (2.4.1).

pH (2.4.24). 4.5 to 7.0, determined in a suspension prepared by shaking 0.1 g with 10 ml of *carbon dioxide-free water* for 15 minutes.

Optical rotation (2.4.22). – 1.34° to – 1.27°, determined at 20° in a solution prepared in the following manner. Dissolve a quantity containing 0.2 g of the substance on the dried basis and 5 g of *hexamine* in 10 ml of *1 M hydrochloric acid*, add sufficient *1 M hydrochloric acid* to produce 25 ml and allow to stand for 3 hours, protected from light.

Light absorption. Dissolve 30 mg in sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with *0.1 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 280 nm; absorbance at 280 nm, 0.41 to 0.44.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Solution A. 1.03 per cent w/v solution of *hydrochloric acid*.

Test solution. Dissolve 0.1 g of the substance under examination in solution A and dilute to 25 ml with solution A.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with solution A. Further dilute 5.0 ml of the solution to 100.0 ml with solution A.

Reference solution (b). A solution containing 0.0008 per cent w/v of *tyrosine* (levodopa impurity B), 0.0004 per cent w/v of *3-methoxy-l-tyrosine* (L-isomer of levodopa impurity C) and 0.0008 per cent w/v of the substance under examination in solution A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with spherical di-isobutyloctadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *0.1 M phosphate buffer pH 3.0*,
  B. a mixture of 18 volumes of *methanol* and 85 volumes of *0.1 M phosphate buffer pH 3.0*,
- a gradient programme using the conditions given below,
- flow rate: 1ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
18	90	10
22	0	100
35	0	100
40	90	10

Name	Relative retention time	Correction factor
Levodopa impurity A[1]	0.7	—
Levodopa (Retention time: about 6 minutes)	1.0	—
Levodopa impurity B[2]	2.0	2.2
Levodopa impurity C[3]	3.5	—

[1]((2*S*)-2-amino-3-(2,4,5-trihydroxyphenyl)propanoic acid,

[2]tyrosine,

[3]3-methoxy-DL-tyrosine.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to levodopa impurity B and levodopa is not less than 10.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to levodopa impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of the peak due to levodopa impurity B is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of the peak due to levodopa impurity C is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent) and the sum of areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dilute 5.0 ml of the test solution to 20.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *D-dopa* (levodopa impurity D) in 10.0 ml of the test solution. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve separately 200 mg of *copper acetate* and 387 mg of *N,N- dimethyl-l-phenylalanine* in *water*, mix the 2 solutions and adjust the pH 4.0 to with *acetic acid*; add 50 ml of *methanol* and dilute to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

The relative retention time with reference to levodopa (Retention time: about 6 minutes) for levodopa impurity D is about 0.4.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to levodopa impurity D and levodopa is not less than 5.0.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with test solution, the area of the peak due to levodopa impurity D is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.3 g, dissolve, heating if necessary, in 5 ml of *anhydrous formic acid* and add 25 ml of *anhydrous glacial acetic acid* and 25 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, using 0.1 ml of *crystal violet solution* as indicator and titrating to a green end-point. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01972 g of $C_9H_{11}NO_4$.

Storage. Store protected from light and moisture.

Levodopa Capsules

L-Dopa Capsules

Levodopa Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levodopa, $C_9H_{11}NO_4$.

Usual strengths. 125 mg; 250 mg; 500 mg.

Identification

A. Dissolve as completely as possible a quantity of the contents of the capsules containing 0.5 g of Levodopa in 25 ml of *1 M hydrochloric acid* and filter. Adjusted to pH 3 with *5 M ammonia*, added dropwise with stirring and allow to

stand for several hours, protected from light. Filter, wash the precipitate and dry it at 105°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levodopa IPRS* or with the reference spectrum of levodopa.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

Mobile phase. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water.*

Test solution. Shake a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 ml of *1 M hydrochloric acid* and filter.

Reference solution. A 1 per cent w/v solution of *levodopa IPRS* in *1 M hydrochloric acid.*

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air and spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide.* The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). −41.5° to −38.5°, determined in the following manner. Weigh accurately a quantity of the contents of the capsules containing 1.25 g of Levodopa, shake with 25.0 ml of *0.5 M hydrochloric acid* for 30 minutes, centrifuge and filter the supernatant liquid. To 10.0 ml of the filtrate add 10 ml of a 21.5 per cent w/v solution of *aluminium sulphate*, 20 ml of a 21.8 per cent w/v solution of *sodium acetate* and sufficient *water* to produce 50.0 ml and measure the optical rotation of the resulting solution at 20°. Separately dilute 5.0 ml of the filtrate to 200.0 ml with *0.1 M hydrochloric acid*, mix well and dilute 10.0 ml to 200.0 ml with *0.1 M hydrochloric acid.* Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_9H_{11}NO_4$, in the filtrate taking 142 as the specific absorbance at 280 nm and from the result so obtained calculate the specific optical rotation.

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_9H_{11}NO_4$ taking 141 as the specific absorbance at 280 nm.

Q. Not less than 75 per cent of the stated amount of $C_9H_{11}NO_4$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

Mobile phase. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water.*

Test solution. A solution prepared immediately before use by shaking a quantity of the contents of the capsules containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of *anhydrous formic acid* and *methanol.*

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with *methanol.*

Reference solution (b). A mixture of equal volumes of the test solution and a solution prepared by dissolving 30 mg of *L-tyrosine* in 1 ml of *anhydrous formic acid* and diluting to 100 ml with *methanol.*

Apply to the plate 10 µl of each of the test solution and reference solution (a) and 20 µl of reference solution (b) as bands. Allow the mobile phase to rise 20 cm. After development, dry the plate in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide* and examine the plate immediately. Any secondary band in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows a distinct band, at a higher R_f value than the principal band, which is more intense than the band in the chromatogram obtained with reference solution (a).

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 0.4 g of Levodopa, dissolve as completely as possible in 10 ml of *anhydrous formic acid*, add 80 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, using *oracet blue B solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01972 g of $C_9H_{11}NO_4$.

Storage. Store protected from light and moisture.

Levodopa Tablets

L-Dopa Tablets

Levodopa Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levodopa, $C_9H_{11}NO_4$.

Usual strengths. 250 mg; 500 mg.

Identification

A. Dissolve as completely as possible a quantity of the tablet containing 0.5 g of Levodopa in 25 ml of *1 M hydrochloric acid* and filter. Adjusted to pH 3 with *5 M ammonia*, added dropwise with stirring and allow to stand for several hours, protected from light. Filter, wash the precipitate and dry it at 105°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levodopa IPRS* or with the reference spectrum of levodopa.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

Mobile phase. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water.*

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Levodopa with 10 ml of *1 M hydrochloric acid* and filter.

Reference solution. A 1 per cent w/v solution of *levodopa IPRS* in *1 M hydrochloric acid.*

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air and spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). −41.5° to −38.5°, determined in the following manner. Weigh accurately a quantity of the powdered tablets containing 1.25 g of Levodopa, shake with 25.0 ml of *0.5 M hydrochloric acid* for 30 minutes, centrifuge and filter the supernatant liquid. To 10.0 ml of the filtrate add 10 ml of a 21.5 per cent w/v solution of *aluminium sulphate*, 20 ml of a 21.8 per cent w/v solution of *sodium acetate* and sufficient *water* to produce 50.0 ml and measure the optical rotation at 20°. Separately dilute 5.0 ml of the filtrate to 200.0 ml with *0.1 M hydrochloric acid*, mix well and dilute 10.0 ml to 200.0 ml with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_9H_{11}NO_4$, in the filtrate taking 142 as the specific absorbance at 280 nm and from the result so obtained calculate the specific optical rotation.

Dissolution (2.5.2):

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7). Calculate the content of $C_9H_{11}NO_4$ taking 141 as the specific absorbance at 280 nm.

Q. Not less than 75 per cent of the stated amount of $C_9H_{11}NO_4$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose.*

Mobile phase. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water.*

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Levodopa with 10 ml of a mixture of equal volumes of *anhydrous formic acid* and *methanol.*

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with *methanol.*

Reference solution (b). A mixture of equal volumes of the test solution and a solution prepared by dissolving 30 mg of *L-tyrosine* in 1 ml of *anhydrous formic acid* and diluting to 100 ml with *methanol.*

Apply to the plate 10 µl each of the test solution and reference solution (a) and 20 µl of reference solution (b) as bands. Allow the mobile phase to rise 20 cm. Dry the plate in a current of warm air, spray with a freshly prepared mixture of equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide* and examine the plate immediately. Any secondary band in the chromatogram obtained with the test solution is not more intense than the band in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows a distinct band, at a higher R_f value than the principal band, which is more intense than the band in the chromatogram obtained with reference solution (a).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.4 g of Levodopa, dissolve as completely as possible in 10 ml of *anhydrous formic acid*, add 80 ml of anhydrous *glacial acetic acid.* Titrate with *0.1 M perchloric acid,* using *oracet blue B solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01972 g of $C_9H_{11}NO_4$.

Storage. Store protected from light and moisture.

Levodopa and Carbidopa Prolonged-release Tablets

Levodopa and Carbidopa Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not

interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Levodopa and Carbidopa Prolonged-release tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levodopa $C_9H_{11}NO_4$ and carbidopa, $C_{10}H_{14}N_2O_4$.

Usual strengths. Carbidopa 25 mg and Levodopa 100 mg; Carbidopa 50 mg and Levodopa 200 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Protect the solutions from light and maintain them at 2° to 8° until they are injected.

Test solution. Weigh and powder 10 tablets. Disperse suitable quantity of the powder in mobile phase. Filter and dilute a quantity of the filtrate with sufficient of the mobile phase to produce a solution containing 0.0125 per cent w/v of carbidopa and 0.05 per cent w/v of levodopa.

Reference solution (a). A solution containing 0.0001 per cent w/v of *levodopa impurity B IPRS* and 0.0125 per cent w/v of *carbidopa IPRS* in mobile phase.

Reference solution (b). A solution containing 0.000125 per cent w/v of *carbidopa IPRS* and 0.0005 per cent w/v of *levodopa IPRS* in mobile phase.

Reference solution (c). A solution containing 0.0000125 per cent w/v of *carbidopa IPRS* and 0.00005 per cent w/v of *levodopa IPRS* from reference solution (b) in mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- sample temperature: 6°,
- mobile phase: a mixture of 95 volumes of a buffer solution prepared by dissolving 6.0 g of *anhydrous monobasic sodium phosphate* in 1000 ml of *water*, adjusted to pH 2.2 with *orthophosphoric acid* and 5 volumes of *ethanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Levodopa impurity A[1]	0.9	1.43
Levodopa	1.0	—
Methyldopa[2]	1.9	—
Levodopa impurity B[3]	2.1	—
Carbidopa	2.3	—
Dihydroxybenzaldehyde[4]	5.7	0.17
Dihydroxyphenylacetone[5]	6.3	1.0
3-O-Methylcarbidopa[6]	6.9	—

[1]3-(3,4,6-trihydroxyphenyl)alanin, impurity based on label claim of levodopa,

[2]Impurity based on label claim of carbidopa, it is process impurity,

[3]3-methoxy-*L*-tyrosine, impurity based on label claim of levodopa,

[4]3,4-dihydroxybenzaldehyde, impurity based on label claim of carbidopa,

[5]3,4-dihydroxyphenylacetone, impurity based on label claim of carbidopa,

[6](s)-2-hydrazinyl-3-(4-hydroxy-3-methoxyphenyl)-2-methyl-propanoic acid, it is process impurity.

Inject reference solution (a) and (c). The test is not valid unless the resolution between the peaks due to carbidopa and levodopa impurity B is not less than 1.5 obtained with reference solution (a) and the signal to noise ratio is not less than 10 for the carbidopa peak, obtained with reference solution (c).

Inject reference solution (b) and the test solution. Run the chromatogram 6 times the retention times of the carbidopa peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to levodopa impurity A is not more than 0.1 times the area of the levodopa peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any peak corresponding to dihydroxybenzaldehyde is not more than 0.2 times the area of the carbidopa peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the area of any peak corresponding to dihydroxyphenylacetone is not more than the area of the carbidopa peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any other secondary peak due to carbidopa is not more than 0.2 times the area of the carbidopa peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the area of any other secondary peak due to levodopa is not more than 0.2 times the area of the levodopa peak in the chromatogram obtained with reference solution (b) (0.2 per cent) The sum of area of all the secondary peaks is not more than 4 times the area of the levodopa peak in the chromatogram obtained with reference solution (b) (4.0 per cent). Disregard any peak with an area less than 0.05 times the area of carbidopa peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Weigh and finely powder 20 tablets. Disperse a quantity of the powder containing about 10 mg of Carbidopa and 40 mg of Levodopa in a 100.0 ml volumetric flask, add 10.0 ml of *0.1 M orthophosphoric acid*, sonicate for 10 minutes and stir for 30 minutes. Dilute to volume with the *water*, stir for another 20 minutes. Filter through 0.45 µm nylon membrane filter.

Reference solution. Dissolve 5 mg of *carbidopa IPRS* and 20 mg of *levodopa IPRS* in a 50.0 ml volumetric flask, add 5 ml of *0.1 M orthophosphoric acid*, sonicate. Dilute to volume with the *water.*

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.13 volumes of the final volume of a buffer solution prepared by dissolving 0.24 g of *sodium 1-decanesulfonate* in 1000 ml of *water* and 95 volumes of the final volume of a buffer solution prepared by dissolving 11.6 g of *monobasic sodium phosphate* in 1000 ml of *water,* adjusted to pH 2.8 with *orthophosphoric acid.* Dilute with *water* to final volume,
- flow rate: 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between levodopa and carbidopa peaks is not less than 6.0, the tailing factor is not more than 1.5 for carbidopa and levodopa peaks and the relative standard deviation for replicate injections of carbidopa and levodopa is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{10}H_{14}N_2O_4$ and $C_9H_{11}NO_4$ in the tablets.

Storage. Store protected from light and moisture.

Levodopa and Carbidopa Tablets

Co-careldopa Tablets

Levodopa and Carbidopa Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous carbidopa, $C_{10}H_{14}N_2O_4$ and not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levodopa, $C_9H_{11}NO_4$.

Usual strengths. Expressed in the form *x/y* where *x* and *y* are the strengths, in mg, of anhydrous carbidopa and levodopa respectively as 10/100; 25/100; 25/250.

Identification

A. In the Assay, the chromatogram obtained with the test solution shows two principal peaks that correspond to those due to carbidopa and levodopa in the chromatogram obtained with the reference solution.

B. Shake a quantity of the powdered tablets containing 50 mg of Levodopa with 4 ml of *ethanol (95 per cent)* and 1 ml of *1 M sulphuric acid.* Add 2 ml of *cinnamaldehyde*, allow to stand for 20 minutes, add 50 ml of *0.1 M hydrochloric acid*, shake for 2 minutes and allow to stand. Filter the aqueous layer and to 5 ml of the filtrate add 0.1 ml of *ferric chloride test solution*. To half of the solution add an excess of *dilute ammonia solution*; a purple colour is produced. To the remainder add an excess of *sodium hydroxide solution*; a deep red colour is produced.

C. Shake a quantity of the powdered tablets containing 1 mg of anhydrous carbidopa with 5 ml of *0.05 M sulphuric acid* and filter. Add 5 ml of *dimethylaminobenzaldehyde reagent* to the filtrate; a yellow colour is produced.

Tests

Dissolution (2.5.2).
Apparatus No. 1 (Basket),
Medium. 750 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution containing 0.005 per cent w/v of *levodopa IPRS* and 0.00054 per cent w/v of *carbidopa IPRS* in the dissolution medium.

Chromatographic system
- a stainless steel column 20 cm × 4.0 mm, packed with octylsilane bonded to porous silica (10 µm) (Such as Lichrosorb RP8),
- mobile phase: a *0.1 M potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *1 M orthophosphoric acid,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the contents of $C_{10}H_{12}N_2O_4$ and $C_9H_{11}NO_4$ in the medium.

Q. Not less than 75 per cent of the stated amounts of $C_{10}H_{12}N_2O_4$ and $C_9H_{11}NO_4$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Disperse one tablet with 20 ml of *0.1 M phosphoric acid* for 30 minutes, add sufficient *water* to produce 200.0 ml, mix and filter.

Reference solution. Weigh 10 mg of *carbidopa IPRS*, dissolve in 20 ml of *0.1 M orthophosphoric acid* by gentle warming and add sufficient *water* to produce 200.0 ml.

Carry out the chromatographic procedure described under Assay.

Calculate the content of $C_{10}H_{14}N_2O_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 250 mg of Levodopa and 25 mg of Carbidopa, with 50 ml of *0.1 M orthophosphoric acid* for 30 minutes, add sufficient *water* to produce 500.0 ml, mix and filter.

Reference solution. Dissolve 250 mg of *levodopa IPRS* and 25 mg of *carbidopa IPRS* in 50.0 ml of *0.1 M phosphoric acid* by gentle warming and add sufficient *water* to produce 500.0 ml.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octylsilane chemically bonded to porous silica (5 µm),
- mobile phase: mix 950 ml of *sodium dihydrogen phosphate solution (1.162 per cent w/v)* with 1.3 ml of *sodium 1-decanesulfonate solution (0.024 per cent w/v)*, adjusted to pH 2.8 with *orthophosphoric acid* and dilute with *water* to produce 1000 ml,
- flow rate: 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

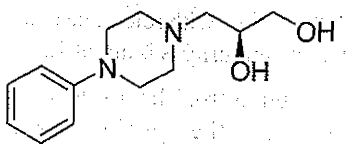
Inject alternately the test solution and the reference solution. The retention times are about 4 minutes and 11 minutes for levodopa and carbidopa respectively.

Calculate the content of $C_{10}H_{14}N_2O_4$ and $C_9H_{11}NO_4$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the quantity of Carbidopa in terms of the equivalent amount of anhydrous carbidopa and the quantity of Levodopa in each tablet.

Levodropropizine



$C_{13}H_{20}N_2O_2$ Mol. Wt. 236.3

Levodropropizine is (2*S*)-3-(4-phenylpiperazin-1-yl)propane-1,2-diol.

Levodropropizine contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{13}H_{20}N_2O_2$, calculated on the dried basis.

Category. Antihistamine.

Description. A white or almost white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levodropropizine IPRS* or with the reference spectrum of levodropropizine.

Tests

Specific optical rotation (2.4.22). – 33.5° to – 30.0°, determined in 3 per cent w/v solution in 2.1 per cent w/v of *hydrochloric acid*.

pH (2.4.24). 9.2 to 10.2, determined in a 2.5 per cent w/v solution in *carbon dioxide-free water*, heat to dissolve and cool.

Impurity B and related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A 0.025 per cent w/v solution of *1-phenylpiperazine IPRS (levodropropizine impurity B IPRS)* in *methanol*. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (b). Mix 1.0 ml of the test solution with 1.0 ml of reference solution (a).

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with end capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 12 volumes of *methanol* and 88 volumes of a 0.68 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,

- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

The relative retention time with reference to levodropropizine for levodropropizine impurity B is about 1.2.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to levodropropizine and levodropropizine impurity B is not less than 2.0.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention times of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to levodropropizine impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurity C. Determine by gas chromatography (2.4.13).

NOTE—Prepare the solutions immediately before use.

Test solution. Dissolve 0.5 g of the substance under examination in *dichloromethane* and dilute to 2.5 ml with *dichloromethane*.

Reference solution (a). A 0.2 per cent w/v solution of *[(2RS)-oxiran-2-yl]methanol IPRS (levodropropizine impurity C IPRS)* in *dichloromethane*. Dilute 0.5 ml of the solution to 100.0 ml with *dichloromethane*.

Reference solution (b). Dissolve 0.5 g of the substance under examination in *dichloromethane*, add 250 µl of reference solution (a) and dilute to 2.5 ml with *dichloromethane*.

Chromatographic system

- a fused-silica column 30 m x 0.53 mm, packed with poly[(cyanopropyl)(phenyl)][dimethyl]siloxane (film thickness 3.0 µm),
- temperature:
  column.140°,
  inlet port. 170° and detector at 250°,
- a flame ionisation detector,
- flow rate: 2.5 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of reference solution (b) and the test solution.

Inject reference solution (b). Run the chromatogram 2.5 times the retention time of levodropropizine impurity C peak. The test is not valid unless the signal-to-noise ratio for levodropropizine impurity C peak is not less than 10.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (5 ppm).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *ethanol* and 60 volumes of *hexane*.

Test solution. Dissolve 10 mg of the substance under examination in 10.0 ml of the solvent mixture. Dilute 1.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.002 per cent w/v solution of *levodropropizine IPRS* in the solvent mixture.

Reference solution (b). A 0.002 per cent w/v solution of *(2R)-3-(4-phenylpiperazin-1-yl)propane-1,2-diol (dextrodropropizine) IPRS (levodropropizine impurity A IPRS)* in the solvent mixture.

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 50.0 ml with the solvent mixture.

Reference solution (d). Dilute 0.5 ml of reference solution (b) to 25 ml with reference solution (a).

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with silica gel OD for chiral separations,
- mobile phase: a mixture of 0.2 volume of *diethylamine*, 5 volumes of *ethanol* and 95 volumes of *hexane*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks corresponding to levodropropizine impurity A and levodropropizine is not less than 1.3.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to levodropropizine impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

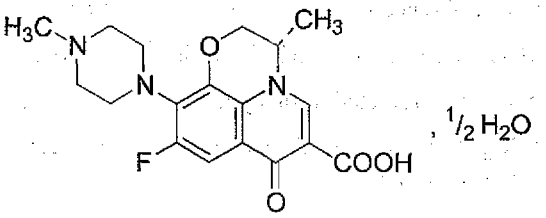
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.25 kPa for 4 hours.

Assay. Dissolve 0.1 g in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01182 g of $C_{13}H_{20}N_2O_2$.

Storage. Store protected from light.

Levofloxacin Hemihydrate



$C_{18}H_{20}FN_3O_4, ½ H_2O$ Mol. Wt. 370.4

Levofloxacin Hemihydrate is (*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid hemihydrate.

Levofloxacin Hemihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{20}FN_3O_4$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A yellowish white to yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levofloxacin hemihydrate IPRS* or with the reference spectrum of levofloxacin hemihydrate.

Tests

Specific optical rotation (2.4.22). – 106° to – 92°, determined in a 0.5 per cent w/v solution in *methanol*, at 20°.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *levofloxacin hemihydrate IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). A 0.00003 per cent w/v solution of *levofloxacin hemihydrate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of buffer solution prepared by dissolving 8.5 g of *ammonium acetate*, 1.25 g of *cupric sulphate pentahydrate* and 1.3 g of *l-isoleucine* in *water* and diluting to 1000 ml with *water*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 360 nm,
- injection volume: 25 µl.

Name	Relative retention time	Correction factor
N-Desmethyl levofloxacin[1]	0.47	—
Diamine derivative[2]	0.52	1.11
Levofloxacin N-oxide[3]	0.63	0.9
9-Desfluoro levofloxacin[4]	0.73	—
Levofloxacin	1.0	—
D-Isomer[5]	1.23	—

[1](S)-9-fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid,

[2](S)-9-fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino)ethylamino]-7-oxo-7H -pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid,

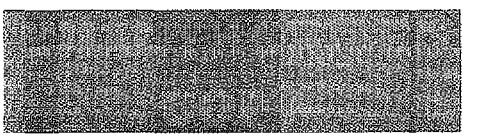
[3](S)-4-(6-carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7H-pyrido-[1,2,3-de][1,4]benzoxazine-10-yl)-1-methyl-piperazine-1-oxide,

[4](S)-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid,

[5](R)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

Inject reference solution (a) and (c). The test is not valid unless the relative standard deviation for replicate injections obtained with reference solution (a) is not more than 1.0 per cent and the signal to noise ratio for the principal peak in the chromatogram obtained with reference solution (c) is not less than 10.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to D-isomer is not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent). The area of any other identified peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the area of all the secondary peaks other than D-isomer is not more than 0.5 times the areas of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).



Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on 1.0 g.

Water (2.3.43). 2.0 per cent to 3.0 per cent, determined on 0.2 g.

Assay. Weigh 0.18 g, dissolve in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml *0.1 M perchloric acid* is equivalent of 0.03614 g of $C_{18}H_{20}FN_3O_4$.

Storage. Store protected from light.

Levofloxacin Infusion

Levofloxacin Infusion is a sterile solution of levofloxacin in 5 per cent dextrose injection or in 0.9 per cent sodium chloride injection.

Levofloxacin Infusion contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of levofloxacin, $C_{18}H_{20}FN_3O_4$.

Usual strength. 5 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution.

Tests

pH (2.4.24). 3.8 to 5.8.

Other tests. Comply with the tests stated under Parenteral Preparation (Infusions).

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and carry out the test protected from light.

Test solution. Measure accurately a volume containing 50 mg of levofloxacin, dilute to 50.0 ml with *0.1 M hydrochloric acid*. Dilute 5.0 ml of the solution to 25.0 ml with *water*.

Reference solution. A 0.1 per cent w/v solution of *levofloxacin hemihydrate IPRS* in *0.1 M hydrochloric acid*. Dilute 5.0 ml of the solution to 25.0 ml with a *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of buffer solution prepared by mixing 84 volumes of *0.05 M citric acid monohydrate* and 1 volume of *1 M ammonium acetate* and 15 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 293 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and column efficiency is not less than 3000 theoretical plates and the relative standard deviation is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{20}FN_3O_4$ in the infusion.

Storage. store protected from light.

Labelling. The label state the strength in terms of equivalent amount of levofloxacin.

Levofloxacin Injection

Levofloxacin Injection is a sterile solution of levofloxacin in Water for Injections.

Levofloxacin Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of levofloxacin, $C_{18}H_{20}FN_3O_4$.

Usual strengths. 25 mg per ml; 50 mg per ml.

Description. A clear, yellowish colour solution.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.8 to 5.8.

Appearance of solution. A 2.5 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensly coloured than reference solution GYS1 (2.4.1).

Light absorption. The absorbance of 0.25 per cent w/v solution in *carbon dioxide-free water* at 420 nm (2.4.7) is not more than 0.6.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and carry out the test protected from light.

Solvent mixture. 60 volumes of buffer solution containing 0.73 per cent w/v of *sodium perchlorate monohydrate*, 0.44 per cent w/v of *ammonium acetate* in *water*, adjusted to pH 2.2 with *orthophosphoric acid* and 40 volumes of *acetonitrile*.

Test solution. Dilute a volume of injection containing 25 mg of Levofloxacin to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.025 per cent w/v solution of *levofloxacin IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of buffer solution and 15 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume: 10 µl.

The retention time of levofloxacin peak is about 8 minutes.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Bacterial endotoxins (2.2.3). Not more than 2.0 Endotoxin Units per mg of Levofloxacin.

Other tests. Comply with the tests stated under Parenteral Preparation (Injections).

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and carry out the test protected from light.

Test solution. Dilute a volume containing 50 mg of Levofloxacin, to 50.0 ml with *0.1 M hydrochloric acid*. Dilute 5.0 ml of the solution to 25.0 ml with *water*.

Reference solution. A 0.1 per cent w/v solution of *levofloxacin IPRS* in *0.1 M hydrochloric acid*. Dilute 5.0 ml of the solution to 25.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of buffer solution prepared by mixing 84 volumes of *0.05 M citric acid monohydrate* and 1 volume of *1 M ammonium acetate* and 15 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 293 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{20}FN_3O_4$ in the injection.

Storage. Store protected from light, at a temperature not exceeding 30°.

Labelling. The label state the strength in terms of equivalent amount of levofloxacin.

Levofloxacin Oral Solution

Levofloxacin Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of levofloxacin, $C_{18}H_{20}FN_3O_4$.

Usual strength. 125 mg per 5 ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 5.0 to 6.0.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay with the following modifications.

Name	Relative retention time	Correction factor
9-desfluorolevofloxacin[1]	0.64	—
Diamine derivative[2]	0.75	—
Levofloxacin impurity A[3]	0.91	1.23
Levofloxacin	1.0	—
Levofloxacin *N*-oxide[4]	1.55	1.07

[1](*S*)-2,3-Dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H* -pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,

[2](*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-[2-(methylamino) ethylamino]-7-oxo-7*H* -pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid,

[3](*S*)-9-Fluoro-2,3-dihydro-3-methyl-10-(piperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carbocylic acid,

[4](*S*)-4-(6-Carboxy-9-fluoro-2,3-dihydro-3-methyl-7-oxo-7*H* -pyrido-[1,2,3-*de*][1,4]benzoxazine-10-yl)-1-methylpiperazine 1-oxide.

Inject the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to levofloxacin impurity A and levofloxacin N-oxide is not more than 0.5 per cent each. The area of any other secondary peak

is not more than 0.2 per cent. The sum of areas of all the secondary peaks is not more 1.0 per cent calculated by area normalization.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14)

NOTE — Protect the solutions from light.

Solvent mixture. 18 volumes of *acetonitrile* and 82 volumes of *water.*

Test solution. Dilute a weighed quantity of the Oral Solution containing about 50 mg of Levofloxacin to 50.0 ml with solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (a). A solution containing about 0.01 per cent of *levofloxacin hemihydrate IPRS* in the solvent mixture.

Reference solution (b). A solution each of 0.01 per cent of *levofloxacin hemihydrate IPRS and levofloxacin impurity A IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, with phenyl groups bonded to porous silica (3.5 µm),
- mobile phase: a mixture of 82 volumes of *water,*18 volumes of *acetonitrile* and 1.0 ml of *trifluoroacetic acid,*
- flow rate: 0.7 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume: 20 µl.

Inject reference solution (a) and (b). Run the chromatogram 2.5 times the retention time of the principal peak. The test is not valid unless the resolution between levofloxacin impurity A and levofloxacin is not less than 1.9 obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{20}FN_3O_4$ in the oral solution.

Determine the weight per ml of the preparation (2.4.29) and calculate the content of $C_{18}H_{20}FN_3O_4$ weight in volume.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label state the strength in terms of equivalent amount of levofloxacin.

Levofloxacin Tablets

Levofloxacin Tablets contain Levofloxacin hemihydrate.

Levofloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of levofloxacin, $C_{18}H_{20}FN_3O_4$.

Usual strengths. 250 mg; 500 mg; 750 mg.

Identification

In the Assay, the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution.

Tests

Dissolution (2.5.2).
Apparatus No. 1 (Basket),
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 293 nm (2.4.7). Calculate the content of $C_{18}H_{20}FN_3O_4$ in the medium from the absorbance obtained from a solution of known concentration of *levofloxacin hemihydrate IPRS* in the same medium.

Q. Not less than 70.0 per cent of the stated amount of $C_{18}H_{20}FN_3O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and carry out the test protected from light.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powdered tablet containing 100 mg of Levofloxacin, disperse in 100.0 ml of *0.1 M hydrochloric acid* and filter. Dilute 5 ml of the solution to 10.0 ml with *water.*

Reference solution (a). A 0.1 per cent w/v solution of *levofloxacin hemihydrate IPRS* in *0.1 M hydrochloric acid.* Dilute 5 ml of the solution to 10 ml with a *water.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water.*

Chromatographic system as described under Assay.

Inject reference solution (a). Test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 3.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Other tests. Comply with the tests stated under the Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and carry out the test protected from light.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powdered tablet containing 100 mg of levofloxacin, disperse in 100.0 ml of *0.1 M hydrochloric acid* and filter. Dilute 5.0 ml of the solution to 50.0 ml with *water.*

Reference solutionn. A 0.1 per cent w/v solution of *levofloxacin hemihydrate IPRS* in *0.1 M hydrochloric acid.* Dilute 5 ml of the solution to 50 ml with *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Cosmosil C18 MS II),
- mobile phase: a mixture of 85 volume of buffer solution prepared by dissolving 84 volumes of *0.05 M citric acid monohydrate* and 1 volume of *1 M ammonium acetate*, filter and 15 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 293 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, column efficiency is not less than 3000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

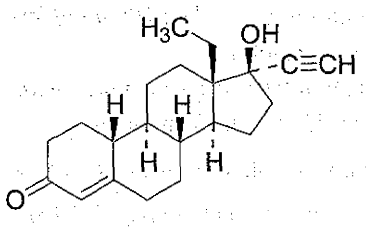
Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{20}FN_3O_4$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label state the strength in terms of equivalent amount of levofloxacin.

Levonorgestrel



$C_{21}H_{28}O_2$ Mol. Wt. 312.5

Levonorgestrel is 13β-ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one.

Levonorgestrel contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{28}O_2$, calculated on the dried basis.

Category. Progestogen.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levonorgestrel IPRS* or with the reference spectrum of levonorgestrel.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum only at about 240 nm.

C. Melts at about 237° (2.4.21).

Tests

Specific optical rotation (2.4.22). −35.0° to −30.0°, determined in a 1.0 per cent w/v solution in *dichloromethane.*

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of *water* and 70 volumes of *acetonitrile.*

Test solution. Dissolve 10 mg of the substance under examination in 7 ml of *acetonitrile* with the aid of ultrasound and dilute to 10.0 ml with *water.*

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octylsilane bonded to porous silica with polar incorporated groups (5 µm),
- mobile phase: A. a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 0.7 ml per minute,
- spectrophotometer set at 215 nm and for impurity O at 200 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
50	20	80
55	100	0

Name	Relative retention time	Correction factor
Levonorgestrel impurity H[1]	0.5	—
Levonorgestrel impurity U[2]	0.8	—
Levonorgestrel impurity K[3]	0.85	—
Levonorgestrel impurity A[4]	0.91	0.4
Levonorgestrel impurity M[5]	0.95	3.1
Levonorgestrel (Retention time: about 20 minutes)	1.0	—
Levonorgestrel impurity O[6]	1.16	2.6
Levonorgestrel impurity B[7]	1.26	—
Levonorgestrel impurity S[8]	1.9	—

[1]6β-hydroxylevonorgestrel,

[2]norethisterone,

[3]18-methylnandrolone,

[4]13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,8(14)-dien-20-yn-3-one,

[5]13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,6-dien-20-yn-3-one,

[6]4,5- dihydro-5α-methoxylevonorgestrel,

[7]13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-5(10)-en-20-yn-3-one,

[8]13-ethyl-3-methoxy-18,19-dinor-17α-pregna-3,5-dien-20-yn-17-ol.

Inject the reference solution. The test is not valid unless the signal-to-noise ratio is not less than 60 for the principal peak in the chromatogram obtained with reference solution.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent). The area of any peak due to levonorgestrel impurity O at 200 nm is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent). The sum of the areas of all the secondary peaks other than levonorgestrel impurity O is not more than 10 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.3 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.1 g, dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml, dilute stepwise with *ethanol (95 per cent)* to obtain a solution containing 0.001 per cent w/v of Levonorgestrel and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7).

Calculate the content of $C_{21}H_{28}O_2$ from the absorbance obtained with a 0.001 per cent w/v solution of *levonorgestrel IPRS* in *ethanol (95 per cent)*.

Storage. Store protected from light and moisture.

Levonorgestrel Tablets

Levonorgestrel Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of levonorgestrel, $C_{21}H_{28}O_2$.

Usual strengths. 30 µg; 750 µg; 1.5 mg.

Identification

Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *water* and 80 volumes of *methanol*.

Test solution. Weigh a quantity of powdered tablets containing 0.15 mg of Levonorgestrel, disperse in 25 ml of the solvent mixture, heat on a water-bath at 60° for 10 minutes, cool and filter.

Reference solution (a). A 0.0006 per cent w/v solution of *levonorgestrel IPRS* in the solvent mixture.

Reference solution (b). A 0.0012 per cent w/v solution of *norgestrel IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of 1.0 per cent w/v solution of *gamma-cyclodextrin*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to levonorgestrel and dextronorgestrel is not less than 1.0.

Inject reference solution (a) and the test solution. The principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

For tablets containing less than 100 µg of levonorgestrel —

Apparatus No. 2 (Paddle),

Medium. 500 ml of *0.01 M hydrochloric acid*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. Use the filtrate, dilute if necessary, with 0.1 per cent w/v solution of *sodium dodecyl sulphate* in dissolution medium to produce a solution containing 0.000006 per cent w/v of levonorgestrel.

Reference solution. A 0.006 per cent w/v solution of *levonorgestrel IPRS* in *methanol*. Dilute 1.0 ml of the solution to 100.0 ml with the dissolution medium. Dilute 1.0 ml of the solution to 10.0 ml with the dissolution medium.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS2),

- mobile phase: a mixture of equal volumes of *acetonitrile* and *water*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 500 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{28}O_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{28}O_2$.

For tablets containing 100 µg or more of levonorgestrel —

Apparatus No. 2 (Paddle),
Medium. 500 ml of 0.1 per cent w/v solution of *sodium lauryl sulphate* in *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A 0.015 per cent w/v solution of *levonorgestrel IPRS* in *methanol*. Dilute 1.0 ml of the solution to 100.0 ml with the dissolution medium.

Use the chromatographic system as described under Dissolution (for tablets containing less than 100 µg of levonorgestrel) with the following modification.

- injection volume: 25 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{28}O_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{28}O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *water* and 50 volumes of *methanol*.

Test solution. Weigh a quantity of powdered tablets containing 0.18 mg of Levonorgestrel, disperse in 5 ml of the solvent mixture, with the aid of ultrasound for 30 minutes, stir vigorously for 15 minutes centrifuge and use the supernatant liquid.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). A 0.004 per cent w/v solution of *ethinylestradiol IPRS* and *levonorgestrel IPRS* in the solvent mixture.

Reference solution (c). Dilute 1.0 ml of the reference solution (a) to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *methanol*, 24 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 200 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to ethinylestradiol and levonorgestrel is not less than 12.0.

Inject reference solution (a) and the test solution. The area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). The sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Uniformity of content. Complies with the test stated under Tablets, as described under Assay using the following solution as test solution.

Test solution. Disperse one tablet in 5 ml of the mobile phase with the aid of ultrasound for 45 minutes and dilute to 10 ml with the mobile phase and centrifuge. Dilute the supernatant liquid with the mobile phase to get concentration of 0.0006 per cent w/v of levonorgestrel.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a sufficient quantity of the powdered tablets and disperse in the mobile phase with the aid of ultrasound, filter and dilute if necessary to get concentration of 0.0006 per cent w/v of levonoregestrel.

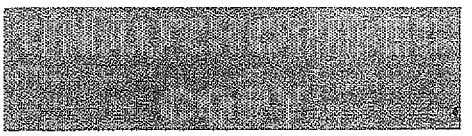
Reference solution. A 0.0006 per cent w/v solution of *levonorgestrel IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of equal volumes of *acetonitrile* and *water*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 25 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{28}O_2$ in the tablets.

Storage. Store protected from light and moisture.



Levonorgestrel and Ethinyloestradiol Tablets

Levonorgestrel and Ethinyloestradiol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of levonorgestrel, $C_{21}H_{28}O_2$ and ethinylo-estradiol, $C_{20}H_{24}O_2$.

Category. Oral contraceptive.

Usual strengths. Levonorgestrel, 150 µg and Ethinyloestradiol, 30 µg; Levonorgestrel, 250 µg and Ethinyloestradiol, 30 µg; Levonorgestrel, 250 µg and Ethinyloestradiol, 50 µg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*

Mobile phase. A mixture of 96 volumes of *dichloromethane* and 4 volumes of *ethanol (95 per cent)*.

Test solution. Powder 20 tablets finely, triturate with 20 ml of *dichloromethane*, allow the solids to sediment and use the clear supernatant liquid.

Reference solution. A solution containing 0.06 per cent w/v of *levonorgestrel IPRS* and 0.006 per cent w/v of *ethinyloestradiol IPRS* in *dichloromethane*.

Apply to the plate 40 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (80 per cent v/v)*, heat at 110° for 10 minutes and examine under ultraviolet light at 365 nm. The principal spots in the chromatogram obtained with the test solution correspond to the spots for levonorgestrel (red fluorescence) and ethinylo-estradiol (orange-yellow fluorescence) in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of 0.0005 per cent w/v solution of *polysorbate 80*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *levonorgestrel IPRS* and *ethinylestradiol IPRS* in the dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

NOTE — A volume of ethanol (95 per cent) not exceeding 2 per cent of the total volume may be used to facilitate the dissolution of reference solution.

Chromatographic system
- a stainless steel column 15 cm x 4 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Zorbax C8),
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 247 nm for levonorgestrel, connected in series with a spectrofluorometre for ethinylestradiol, with an excitation wavelength of 285 nm and an emission wavelength of 310 nm,
- injection volume: 100 µl.

The relative retention time with respect to levonorgestrel for ethinylestradiol is about 0.7.

Inject the reference solution. The relative standard deviation for replicate injections is not more than 3.0 per cent for both the peaks.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{21}H_{28}O_2$ and not less than 75 per cent of the stated amount of $C_{20}H_{24}O_2$.

Uniformity of content. Complies with the test stated under Tablets.

Carry out the procedure described under Assay using the following solution.

Test solution. Disperse one tablet in 5 ml of mobile phase with the aid of ultrasound for 30 minutes, cool and dilute to 10.0 ml with the mobile phase. Shake to mix and centrifuge, use the clear supernatant liquid.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 1.5 mg of Levonorgestrel with 75 ml of mobile phase with the aid of ultrasound for 30 minutes, cool and dilute to 100.0 ml with the mobile phase. Shake to mix and centrifuge, use the clear supernatant liquid.

Reference solution (a). A 0.015 per cent w/v solution of *levonorgestrel IPRS* in mobile phase.

Reference solution (b). A 0.003 per cent w/v solution of *ethinylostradiol IPRS* in mobile phase.

Reference solution (c). Dilute reference solution (a) and reference solution (b) with mobile phase to obtain a solution having a concentration similar to the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile*, 15 volumes of *methanol* and 45 volumes of *water*,
- flow rate: 1 to 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

Inject reference solution (c). The test is not valid unless the resolution between the two major peaks is not less than 2.5 and the relative standard deviation for replicate injections is not more than 2.0.

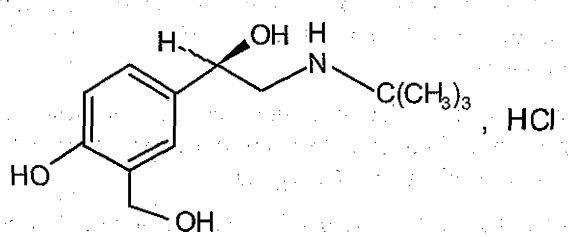
Inject reference solution (c) and the test solution. The relative retention time with reference to levonorgestrel for ethinyloestradiol is about 0.7.

Calculate the contents of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ in the tablets.

Storage. Store protected from moisture.

Levosalbutamol Hydrochloride

R- Albuterol Hydrochloride



$C_{13}H_{21}NO_3,HCl$ Mol.Wt. 275.8

Levosalbutamol Hydrochloride is (R) α^1-[(tert-Butylamino)methyl]-4-hydroxy-m-xylene- α, α^1-diol hydrochloride.

Levosalbutamol Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{13}H_{21}NO_3$, HCl, calculated on the anhydrous basis.

Category. Bronchodialator.

Description. A white to off white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *levosalbutamol hydrochloride IPRS* or with the reference spectrum of levosalbutamol hydrochloride.

Tests

pH (2.4.24). 4.5 to 5.5, determined on 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in mobile phase A and dilute to 100.0 with mobile phase A.

Reference solution. A 0.0001 per cent w/v solution of *levosalbutamol hydrochloride IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: A. a 0.1 per cent v/v solution of *orthophosphoric acid* in *water*,
  B. a mixture of 35 volumes of *acetonitrile*, 35 volumes of *methanol*, 0.1 volume of *orthophosphoric acid* and 30 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile Phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0	100	0
30	70	30
50	28	72
50.1	0	100
55	0	100
55.1	100	0
70	100	0

Name	Relative retention time	Correction factor
Levosalbutamol	1.0	–
Levosalbutamol impurity D[1]	1.7	0.3
Levosalbutamol impurity F[2]	3.5	0.8

[1]5-[2-{(1,1-dimethylethyl)amino}-1-hydroxyethyl]-2-hydroxy-benzaldehyde benzenesulphonic acid,

[2]á[{(1,1-dimethylethyl)amino}methyl]-4-(phenylmethoxy)-1,3-benzenedimethanol.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 4.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	91.5	8.5
15	91.5	8.5
15.1	0	100
20	0	100
20.1	91.5	8.5
30	91.5	8.5

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

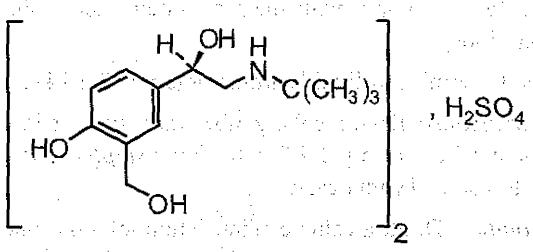
Calculate the content of $C_{13}H_{21}NO_3$ in the inhalation solution.

Storage. Store protected from light, at a temperature not exceeding 30°.

Labelling. The label indicates the dose and that the ampoules should be discarded if the solution is not colourless.

Levosalbutamol Sulphate

(*R*)-Albuterol Sulphate



$(C_{13}H_{21}NO_3)_2.H_2SO_4$ Mol. Wt. 576.7

Levosalbutamol Sulphate is (*R*)-2-*tert*-butylamino-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol sulphate.

Levosalbutamol Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{13}H_{21}NO_3)_2.H_2SO4$, calculated on the dried basis.

Category. β_2-adrenoreceptor agonist.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrphotometry (2.4.6). Compare the spectrum with that obtained with *levosalbutamol sulphate IPRS* or with the reference spectrum of levosalbutamol sulphate.

B. When examined in the range 200 to 350 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* shows absorption maxima at about 227 nm and 278 nm.

Tests

Specific optical rotation (2.4.22). −40° to −30°, determined on 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 100 mg of the substance under examination in 50 ml of the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *levosalbutamol sulphate IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Waters symmetry C8),
- mobile phase. a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.29 per cent w/v of *sodium heptanesulphonate* and 0.25 per cent w/v of *potassium dihydrogen phosphate*, adjusted to pH 3.7 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 10 mg of the substance under examination in 4 ml of methanol and dilute to 10.0 ml with the mobile phase.

Reference solution. Dissolve 10 mg of *Salbutamol sulphate IPRS* in 4 ml of *methanol* and dilute to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, bonded to porous silica (5 µm), (Such as Chirex 3022),

- mobile phase. a mixture of 70 volumes of *n-hexane*, 23 volumes of *dichloroethane*, 7 volumes of *methanol* and 0.1 volume of *trifluoroacetic acid*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to levosalbutamol and dextrosalbutamol is not less than 1.0. The first peak is due to dextrosalbutamol and the second peak is due to levosalbutamol.

Inject the reference solution and the test solution. Run the chromatogram for 60 minutes. The content of the peak due to (S)-salbutamol sulphate is not more than 1.0 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 50 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *levosalbutamol sulphate IPRS* in the mobile phase.

Chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the theoretical plates for the principal peak is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

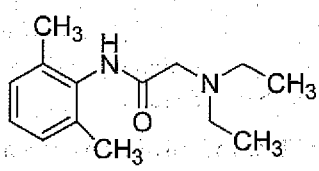
Inject the reference solution and the test solution.

Calculate the content of $(C_{13}H_{21}NO_3)_2.H_2SO_4$.

Storage. Store at a temperature not exceeding 30°.

Lignocaine

Lidocaine



$C_{14}H_{22}N_2O$ Mol. Wt. 234.3

Lignocaine is acetamide, 2-(diethylamino)-*N*-(2,6-dimethylphenyl)-; 2-(Diethylamino)-2',6'-acetoxylidide.

Lignocaine contains not less than 97.5per cent and not more than 102.5 per cent of $C_{14}H_{22}N_2O$.

Category. Local anaesthetic.

Description. A white or slightly yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lignocaine IPRS* or with the reference spectrum of lignocaine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Melting range (2.4.21). 66° to 69°.

Tests

Chlorides (2.3.12). Dissolve 7.14 g of the substance under examination in *water* add 10 ml of dilute *nitric acid*, dilute to 50 ml with *water*. The resulting solution complies with the limit test for chlorides (35 ppm).

Sulphates (2.3.17). 0.15 g complies with the limit test of sulphates (1000 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 85 mg of the substance under examination with heating, if necessary in 0.5 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dissolve 85 mg *lignocaine IPRS* with heating if necessary in 0.5 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with the mobile phase.

Reference solution (b). A 0.022 per cent w/v solution of *methylparaben* in the mobile phase.

Reference solution (c). A solution containing 2 volumes of reference solution (b) and 20 volumes of reference solution (a).

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of a solution prepared by diluting 50 volumes of *glacial acetic acid* to 980 volumes of *water*, adjusted to pH 3.4 with *1M sodium hydroxide* and 20 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (b) and (c). The test is not valid unless the resolution between the peaks due to lignocaine and methylparaben is not less than 3.0 obtained with reference solution (c) and the relative standard deviation for replicate injections is not more than 1.5 per cent obtained with reference solution (b)

Inject reference solution (b) and the test solution.

Calculate the content of $C_{14}H_{22}N_2O$.

Storage. Store at a temperature not exceeding 30°

Lignocaine Oral Topical Solution

Lidocaine Oral Topical solution

Lignocaine Oral Topical Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lignocaine, $C_{14}H_{22}N_2O$. It contains a suitable flavour.

Usual strengths. 2 per cent w/v; 4 per cent w/v.

Identification

Transfer a quantity of Oral Topical Solution, containing 250 mg of lignocaine, to a separator with 20 ml of *water* and extract with 20 ml of *chloroform*. Wash the *chloroform* extract with 20 ml of *water* and evaporate the *chloroform* extract to dryness in a current of warm air. Dissolve the residue in *hexane*, evaporate in a current of warm air and dry the residue in vacuum over silica gel for 24 hours, crystalline precipitate is produced. The precipitate complies with the following tests.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lignocaine IPRS* or with the reference spectrum of lidocaine.

Tests

Other tests. Comply with the tests stated under Oral Solution.

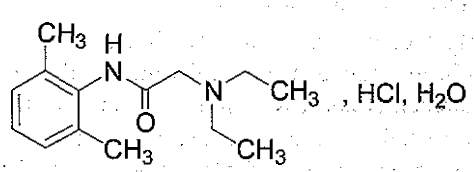
Assay. Transfer an accurately measured volume containing about 0.15 g of lignocaine to a 125 ml conical flask and protect from atmospheric moisture with a stopper fitted with a tube containing silica gel. Add 20 ml of *glacial acetic acid*. Titrate immediately with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02343 g of $C_{14}H_{22}N_2O$.

Storage. Store protected from moisture.

Lignocaine Hydrochloride

Lidocaine Hydrochloride



$C_{14}H_{22}N_2O,HCl,H_2O$ Mol. Wt. 288.8

Lignocaine Hydrochloride is 2-diethylaminoaceto-2′,6′-xylidide hydrochloride monohydrate.

Lignocaine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{22}N_2O,HCl$, calculated on the anhydrous basis.

Category. Local anaesthetic; antiarrhythmic.

Description. A white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lignocaine hydrochloride IPRS* or with the reference spectrum of lignocaine hydrochloride.

B. To 10 ml of a 2.5 per cent w/v solution add *sodium hydroxide solution* till alkaline and filter. Wash the residue with *water*, dissolve half of the residue in 1 ml of *ethanol (95 per cent)* and add 0.5 ml of a 10 per cent w/v solution of *cobalt chloride*; a bluish-green precipitate is produced.

C. To 5 mg add 0.5 ml of *fuming nitric acid*, evaporate to dryness on a water-bath, cool, dissolve the residue in 5 ml of *acetone* and add 1 ml of *0.1 M ethanolic potassium hydroxide*; a green colour is produced.

D. Dissolve 0.2 g in 10 ml of *water* and add 10 ml of *picric acid solution*. The precipitate, after washing with *water* and drying, melts at about 229° (2.4.21).

E. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 4.0 to 5.5, determined in a 0.5 per cent w/v solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Sulphates. Dissolve 0.2 g in 20 ml of *water*, add 2 ml of *3 M hydrochloric acid*, mix and divide into two parts. To one part add 1 ml of *barium chloride solution*; no more opalescence is produced than in the remaining portion of the solution to which nothing has been added.

2,6-Dimethylaniline. To 2 ml of a 2.5 per cent w/v solution in *methanol* (solution A), add 1 ml of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand for 10 minutes at room temperature. Any yellow colour produced is more intense than

that obtained by repeating the test using 2 ml of *methanol* in place of solution A and less intense than the colour produced using a mixture of 1 ml of a solution of *2,6-dimethylaniline* in *methanol* containing 5 µg per ml and 1 ml of *methanol* in place of solution A (100 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 5.0 to 7.5 per cent, determined on 0.25 g.

Assay. Weigh 0.5 g, dissolve in 30 ml of *anhydrous glacial acetic acid*, add 6 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02708 g of $C_{14}H_{22}N_2O,HCl$.

Lignocaine Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.1 Endotoxin Units per mg of lignocaine hydrochloride.

Storage. Store protected from moisture.

Lignocaine Gel

Lignocaine Hydrochloride Gel; Lidocaine Hydrochloride Gel

Lignocaine Gel is a sterile solution of Lignocaine Hydrochloride in a suitable water-miscible base. It may contain suitable antioxidants, stabilisers and antimicrobial preservatives.

Lignocaine Gel contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl$.

Usual strengths. The equivalent of 1 per cent and 2 per cent w/v of anhydrous lignocaine hydrochloride.

Identification

To a quantity of the gel containing 80 mg of anhydrous lignocaine hydrochloride add 4 ml of *hydrochloric acid* and heat on a water-bath for 10 minutes. Allow to cool, transfer to a separating funnel with the aid of 20 ml of *water*, add *5 M sodium hydroxide* until precipitation is complete and extract with two quantities, each of 20 ml, of *chloroform*. Filter the chloroform extracts through *anhydrous sodium sulphate* and evaporate the filtrate to dryness on a water-bath using a stream of nitrogen. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lignocaine hydrochloride IPRS* treated in the same manner or with the reference spectrum of lignocaine.

B. Dissolve 20 mg in 1 ml of *ethanol (95 per cent)*, add 0.5 ml of a 10 per cent w/v solution of *cobalt chloride* and 0.5 ml of *5 M sodium hydroxide* and shake for 2 minutes; a bluish green precipitate is produced.

C. Dissolve 40 mg in 5 ml of 1 per cent w/v solution of *cetrimide*, add 1 ml of *5 M sodium hydroxide* and 1 ml of *bromine water*; a yellow colour is produced.

Tests

pH (2.4.24). 6.0 to 7.0.

2,6-Dimethylaniline. Mix a quantity of the gel containing 15 mg of anhydrous lignocaine hydrochloride with sufficient *water* to produce 3 ml, using a rotary mixer. To 2 ml of the resulting solution, add 1 ml of a freshly prepared 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *methanol*. Mix thoroughly using a rotary mixer. Add 2 ml of *glacial acetic acid* and allow to stand for 10 minutes. Any yellow colour produced is not more intense than that obtained by using a mixture of 2 ml of a solution of *2,6-dimethylaniline* in *methanol* containing 2 µg per ml in place of the solution of the gel (400 ppm).

Sterility (2.2.11). Complies with the test for sterility.

Other tests. Comply with the tests stated under Gels.

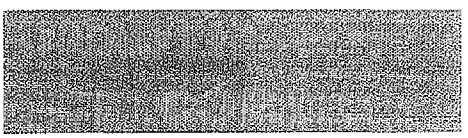
Assay. Weigh accurately a quantity containing about 10 mg of anhydrous lignocaine hydrochloride and disperse in 20 ml of *water*. Add 5 ml of *acetate buffer pH 2.8*, 120 ml of *chloroform* and 5 ml of *dimethyl yellow-oracet blue B solution* and titrate with *0.005 M dioctyl sodium sulphosuccinate* swirling vigorously, until near the end-point, then add the titrant dropwise and, after each addition, swirl vigorously, allow to separate and swirl gently for 5 seconds. The end-point is indicated when the colour of the chloroform layer changes from green to pinkish-grey. Carry out a blank titration.

1 ml of *0.005 M dioctyl sodium sulphosuccinate* is equivalent to 0.001354 g of $C_{14}H_{22}N_2O,HCl$.

Determine the weight per ml of the gel (2.4.29) and calculate the percentage of $C_{14}H_{22}N_2O,HCl$, weight in volume.

Storage. Store in suitable tamper-proof containers holding sufficient of the gel for use on one occasion and at a temperature not exceeding 30°. The gel should not be frozen.

Labelling. The label states (1) that the contents are sterile; (2) the strength in terms of the equivalent amount of anhydrous lignocaine hydrochloride; (3) that any of the gel not used in a single application should be discarded.



Lignocaine Injection

Lignocaine Hydrochloride Injection; Lidocaine Hydrochloride Injection; Lidocaine Injection

Lignocaine Injection is a sterile solution of Lignocaine Hydrochloride in Water for Injections.

Lignocaine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl,H_2O$.

Usual strengths. 0.2 per cent w/v; 1.0 per cent w/v; 5.0 per cent w/v.

Description. A clear colourless solution.

Identification

A. To a volume containing 0.1 g of Lignocaine Hydrochloride add sufficient *sodium hydroxide solution* to make alkaline, filter, wash the residue with *water*, dissolve in 1 ml of *ethanol (95 per cent)*, add 0.5 ml of a 10 per cent w/v solution of *cobalt chloride* and shake for 2 minutes; a bluish-green precipitate is formed.

B. To a volume containing 0.1 g of Lignocaine Hydrochloride add 10 ml of *picric acid solution*; the precipitate, after washing with *water* and drying at 105°, melts at about 229° (2.4.21).

C. It gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.0.

2,6-Dimethylaniline. To a volume containing 25 mg of Lignocaine Hydrochloride add *water* if necessary to produce 10 ml, add *2 M sodium hydroxide* until the solution is just alkaline and extract with three quantities, each of 5 ml, of *chloroform*. Dry the combined chloroform extracts over *anhydrous sodium sulphate*, filter, wash with a further 5 ml of *chloroform* and evaporate the filtrate to dryness at a pressure of 2 kPa. Dissolve the residue in 2 ml of *methanol*, add 1 ml of a 1 per cent w/v solution of *4-dimethylamino-benzaldehyde* in *methanol* and 2 ml of *glacial acetic acid* and allow to stand at room temperature for 10 minutes. Any yellow colour produced is not more intense than the colour produced by repeating the operation using 10 ml of a solution in *water* containing 1 µg per ml of *2,6-dimethylaniline* in place of the preparation under examination (400 ppm).

Bacterial endotoxins (2.2.3). Not more than 1.1 Endotoxin Units per mg of lignocaine hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Make an accurately measured volume containing about 0.1 g of Lignocaine Hydrochloride alkaline with *2 M sodium hydroxide* and extract with three quantities, each of 20 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the washed extracts through a filter paper moistened with *chloroform*, wash the filter with 10 ml of *chloroform*, combine the washings with the filtrate. Titrate with *0.02 M perchloric acid*, using *crystal violet solution* as indicator.

1 ml of *0.02 M perchloric acid* is equivalent to 0.005776 g of $C_{14}H_{22}N_2O,HCl,H_2O$.

Lignocaine Hydrochloride Topical Solution

Lidocaine Hydrochloride Topical solution

Lignocaine Hydrochloride Topical Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl$.

Category. Topical anaesthetic agent.

Usual strengths. 2 per cent w/v; 4 per cent w/v.

Identification

A. Transfer a quantity of topical solution, containing 200 mg of lignocaine hydrochloride, to a separator extract with four 15 ml portions of *chloroform*, discarding the chloroform extracts. Add 2 ml of *2 M sodium hydroxide* to the aqueous solution remaining in the separator and extract with four 15 ml portions of chloroform. Combine the *chloroform* extracts and evaporate the filtrate to dryness on a water-bath using a stream. The residue complies with the following tests.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lignocaine hydrochloride IPRS* treated in the same mnanner or with the reference spectrum of lignocaine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

Tests

pH (2.4.24). 5.0 to 7.0.

Related substances. Determine by liquid chromatography (2.4.14), using solvent mixture and mobile phase as described under Assay.

Test solution. Dissolve 20 mg of lignocaine hydrochloride in 10.0 ml of the mobile phase.

Reference solution (a). A solution containing 0.00026 per cent w/v of *lignocaine IPRS*, 0.00039 per cent w/v of *ropivacaine impurity A IPRS (2,6-dimethylaniline*

hydrochloride) and 0.0003 per cent w/v of *lignocaine impurity H IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.00017 per cent w/v of *lignocaine IPRS*, 0.00026 per cent w/v of *ropivacaine impurity A IPRS* and 0.0002 per cent w/v of *lignocaine impurity H IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Name	Relative retention time
Lignocaine	1.0
Dimethylaniline	3.2
Lidocaine impurity H[1]	3.8

[1]N-(chloroacetyl)-2,6-xylidide.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to lignocaine impurity H and ropivacaine impurity A is not less than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to lidocaine impurity H is not more than the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.1 per cent) and the area of any peak due to ropivacaine impurity A is not more than 0.8 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.1 per cent). The area of any secondary peak is not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 23 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 5 volumes of *glacial acetic acid* and 93 volumes of *water,* adjusted the pH to 3.4 with *1 M sodium hydroxide.*

Test solution. Measure a volume containing 20 mg of Lignocaine Hydrochloride to 10.0 ml with the mobile phase.

Reference solution (a). Transfer 20.0 mg of *lignocaine hydrochloride IPRS* to 10.0 ml volumetric flask, add 0.1 ml of *1 M hydrochloric acid* and dilute to volume with mobile phase.

Reference solution (b). A 0.022 per cent w/v solution of *methylparaben IPRS* in the mobile phase.

Reference solution (c). Dilute 2.0 ml of reference solution (b) to 20 ml with the reference solution (a).

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 40 volumes of solvent mixture.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm
- injection volume: 20 µl.

Inject reference solution (a) and (c). The test is not valid unless the resolution between the peaks due to lignocaine and methylparaben is not less than 3.0 obtained with reference solution (c) and the relative standard deviation for replicate injections is not more than 1.5 per cent obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of the lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl$.

Storage. Store at a temperature not exceeding 30°.

Lignocaine and Adrenaline Injection

Lidocaine Hydrochloride and Adrenaline Bitartrate Injection; Lidocaine and Adrenaline Injection; Adrenaline Bitartrate and Lidocaine Hydrochloride Injection

Lignocaine and Adrenaline Injection is a sterile solution of Lignocaine Hydrochloride and Adrenaline Bitartrate in Water for Injections.

Lignocaine and Adrenaline Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl,H_2O$ and not less than 87.5 per cent and not more than 112.5 per cent of the stated amount of adrenaline, $C_9H_{13}NO_3$.

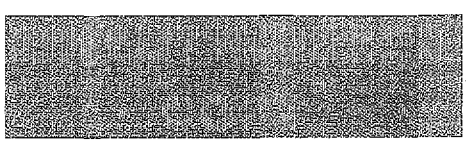
Usual strength. Lignocaine Hydrochloride, 20 mg per ml and adrenaline, 0.01 mg per ml.

Description. A clear colourless solution.

Identification

A. To 5 ml add 1 ml of *hydrochloric acid,* cool to 0°, add 5 ml of a 1 per cent w/v solution of *sodium nitrite* and pour the mixture into 2 ml of *2-naphthol solution* containing 1 g of *sodium acetate*; no red colour is produced.

B. To 10 ml add 4 ml of *disodium hydrogen phosphate solution* and sufficient *0.1 M iodine* to produce a distinct brown colour.



Add *0.01 M sodium thiosulphate* to remove the excess of iodine; a pink colour is produced.

C. To 3 ml add 3 ml of *water* and 6 ml of *picric acid solution*, shake gently and allow to stand until the precipitate becomes crystalline; the precipitate, after washing with *water* and drying at 105°, melts at about 229° (2.4.21).

Tests

pH (2.4.24). 3.0 to 4.5.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *For lignocaine hydrochloride* — Make an accurately measured volume containing 0.1 g of Lignocaine Hydrochloride alkaline with *2 M sodium hydroxide* and extract with three quantities, each of 20 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the washed extracts through a filter paper moistened with *chloroform*, wash the filter with 10 ml of *chloroform*, combine the washings with the filtrate. Titrate with *0.02 M perchloric acid*, using *crystal violet solution* as indicator.

1 ml of *0.02 M perchloric acid* is equivalent to 0.005776 g of $C_{14}H_{22}N_2O,HCl,H_2O$.

For adrenaline — Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 8.0 g of *tetramethylammonium hydrogen sulphate*, 2.2 g of *sodium heptanesulphonate*, add 2 ml of *0.1M disodium edetate* to a mixture of 900 ml of *water* and 100 ml of *methanol* , adjusted to pH 3.5 using *1M sodium hydroxide*.

Test solution. Dilute a volume of injection to obtain a solution containing 0.0005 per cent w/v of adrenaline. Dilute 5.0 ml of the resulting solution to 10 ml with the solvent mixture.

Reference solution (a). Dilute 5.0 ml of a 0.01 per cent w/v solution of *adrenaline acid tartrate IPRS* to 100.0 ml with the solvent mixture.

Reference solution (b). A mixture of 5 ml of reference solution (a) with 5 ml of a 0.001 per cent w/v solution of *noradrenaline acid tartrate* in the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution prepared by dissolving 4.0 g of *tetramethylammonium hydrogen sulphate*, 1.1 g of *sodium heptanesulphonate*, add 2 ml of *0.1M disodium edetate* to a mixture of 950 ml of *water* and 50 ml of *methanol* and adjusted to pH 3.5 with *1M sodium hydroxide*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 2.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_9H_{13}NO_3$.

Storage. Store protected from light.

Labelling. The label states the strength of Adrenaline Bitartrate in terms of the equivalent amount of adrenaline.

Lignocaine and Dextrose Injection

Lignocaine Hydrochloride and Dextrose Injection; Lidocaine Hydrochloride and Dextrose Injection; Lidocaine and Dextrose Injection

Lignocaine and Dextrose Injection is a sterile solution of Lignocaine Hydrochloride and Dextrose in Water for Injections.

Lignocaine and Dextrose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of lignocaine hydrochloride, $C_{14}H_{22}N_2O,HCl,H_2O$ and dextrose, $C_6H_{12}O_6,H_2O$.

Usual strength. Lignocaine Hydrochloride 50 mg per ml and Dextrose 75 mg per ml.

Description. A clear colourless or slightly yellow solution.

Identification

A. To a volume containing about 0.5 g of Lignocaine Hydrochloride in a separator add 2 ml of *2 M sodium hydroxide* and extract with four quantities, each of 15 ml, of *chloroform*. Combine the chloroform extracts and evaporate the solution to dryness with the aid of a current of air. Dissolve the residue in 2 ml of *hexane*, evaporate with the aid of warm air and dry the residue over *silica gel* for 24 hours at a pressure not exceeding 0.7 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lignocaine hydrochloride IPRS* treated in the same manner or with the reference spectrum of lignocaine.

B. To a volume containing 0.1 g of Dextrose add 10 ml of *water* and 3 ml of *potassium cupri-tartrate solution* and heat; a red precipitate is produced.

Tests

pH (2.4.24). 3.0 to 7.0.

Bacterial endotoxins (2.2.3). Not more than 1.1 Endotoxin Units per mg of lignocaine hydrochloride.

Other tests. Comply with the tests described under Parenteral Preparations (Injections).

Assay. *For lignocaine hydrochloride*—Make an accurately measured volume containing about 0.1 g of Lignocaine Hydrochloride alkaline with *2 M sodium hydroxide* and extract with three quantities, each of 20 ml, of *chloroform*, washing each extract with the same 10 ml of *water*. Filter the washed extracts through a filter paper moistened with *chloroform*, wash the filter with 10 ml of *chloroform*, combine the washings with the filtrate. Titrate with *0.02 M perchloric acid*, using *crystal violet solution* as indicator.

1 ml of *0.02 M perchloric acid* is equivalent to 0.005776 g of $C_{14}H_{22}N_2O,HCl,H_2O$.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose add sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 1.0425 represents the weight, in g, of *dextrose*, $C_6H_{12}O_6,H_2O$ in the volume taken for assay.

Storage. Store in single dose containers in a cool place.

Lignocaine and Prilocaine Cream

Lidocaine and Prilocaine Cream

Lignocaine and Prilocaine Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lignocaine, $C_{14}H_{22}N_2O$ and Prilocaine, $C_{13}H_{20}N_2O$.

Usual strength. Lidocaine 2.5 per cent, Prilocaine 2.5 per cent.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 8.7 to 9.7, determine in 1.0 per cent w/v solution or in the undiluted sample.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

NOTE — Store the solution at or below 10°.

Reference solution (c). A solution containing 0.0002 per cent w/v of *lignocaine IPRS and prilocaine hydrochloride IPRS* in mobile phase A.

Name	Relative retention time	Correction factor
o-Toluidine[P][2]	0.38	0.43
n-Chloroacetyl-2,6-xylidine[L][3]	0.54	1.0
2,6-Dimethylaniline[L][3]	0.67	0.30
Prilocaine[1]	1.00	—
2-Diethylaminoaceto-2,4-xylidine[L][3]	1.33	1.25
Lignocaine	2.14	—
n-Dichloroacetyl-2,6-xylidine[L][3]	2.98	0.45
Any other individual related compounds [P][2]	—	1.0
Total related compounds, excluding *o*-toluidine	—	—

[1]Relative to the prilocaine peak,

[2]P designates a prilocaine related compound,

[3]L designates a lidocaine related compound.

Inject reference solution (b) and (c).The test is not valid unless the resolution between the peaks due to prilocaine and prilocaine related compound B is not less than 1.4 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (c).

Inject reference solution (c) and the test solution. In the chromatogram obtained with test solution, the area of any peak corresponding to *o*-toluidine is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent). The area of any peak corresponding to n-chloroacetyl-2,6-xylidine is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The area of any peak corresponding to 2,6-dimethylaniline is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The area of any peak corresponding to 2-diethylaminoaceto-2,4-xylidine is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The area of any peak corresponding to n-dichloroacetyl-2,6-xylidine is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The area of any peak corresponding to any other individual related compounds is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent). The sum of all the secondary peaks excluding *o*-toluidine is not more than the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Store the solution at or below 10°.

Test solution. Disperse a quantity of the cream containing about 20 mg of Lignocaine in 100.0 ml volumetric flask. Add 5 ml of *5M sodium hydroxide* and mix, Add 5 ml of *5M hydrochloric acid* and dilute to 100.0 ml with the mobile phase A and filter.

Reference solution (a). A solution containing each of 0.02 per cent w/v of *lignocaine IPRS and prilocaine hydrochloride IPRS* in the mobile phase A.

Reference solution (b). Dissolve an accurately weighed quantity of *prilocaine related compound B IPRS* in the reference solution and dilute quantitatively and stepwise if necessary, with the reference preparation to obtain a solution having a known concentration of about 0.008 per cent w/v of prilocaine related compound B.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- sample temperature: 10°,
- mobile phase: A. a buffer solution prepared by dissolving 2.73 g of *monobasic potassium phosphate* in 630 ml of *water*, adjusted to pH 7.2 with *5M sodium hydroxide* and dilute to 1000 ml with *acetonitrile*.

  B. a buffer solution prepared by dissolving 2.73 g of *monobasic potassium phosphate* in 900 ml of *water*, adjusted to pH 7.2 with *5M sodium hydroxide* and dilute to 1000 ml with *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 232 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	67	33
11	67	33
22	100	0
32	100	0
32.1	67	33
37	67	33

The relative retention times are 1.0 for prilocaine, 1.09 for prilocaine related compound B and 2.14 for lidocaine.

Inject reference solution (b).The test is not valid unless the resolution between the peaks of prilocaine and prilocaine related compound B is not less than 1.4.

Inject reference solution (a).The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 1.5 based on the prilocaine peak and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

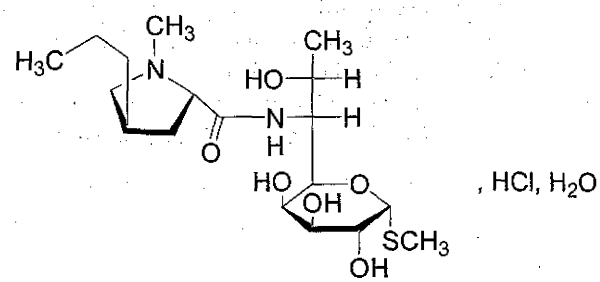
Calculate the contents of $C_{14}H_{22}N_2O$ and $C_{13}H_{20}N_2O$ in the cream.

Storage. Preserve in collapsible tubes or tight containers at a temperature not exceeding 30°. Protect from freezing.

Labelling. The label states the strength of Prilocaine Hydrochloride in terms of the equivalent amount of prilocaine.

Lincomycin Hydrochloride

Lincomycin Hydrochloride Monohydrate



$C_{18}H_{35}ClN_2O_6S, H_2O$ Mol. Wt. 461.0

Lincomycin Hydrochloride consists mainly of methyl6-amino-6,8-dideoxy-6-[[[(2S,4R)-1-methyl-4-propylprrolidin-2-yl]carbonyl]amino]-1-thio-D-erythro-α-D-galacto-octopyranoside(lincomycin)hydrochloridemonohydrate, a mixture of antibiotics producedby *Streptomyces lincolnensis* var. *lincolnensis* or obtained by anyother means.

Lincomycin Hydrochloride contains not less than 96.0 per cent and not more than 102.0 per cent of the sum of Lincomycin Hydrochloride, $C_{18}H_{35}ClN_2O_6S$ and Lincomycin B Hydrochloride, $C_{17}H_{33}ClN_2O_6S$, Lincomycin B Hydrochloride contains not more than 5.0 per cent and calculated on the anhydrous basis.

Category. Antibacterial

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lincomycin hydrochloride IPRS* or with the reference spectrum of lincomycin hydrochloride.

B. A 1 per cent w/v solution gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbondioxide-free water* (Solution A) is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 3.5 to 5.5 determined in solution A.

Specific optical rotation (2.4.22). + 135° to + 150°, determined in 4.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.25 per cent w/v solution of *lincomycin hydrochloride IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). Dissolve 5 mg of *lincomycin hydrochloride for system suitability IPRS* (containing impurities A, B and C) in 2 ml of the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated end capped octylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 34 g of *ortho-phosphoric acid* in 900 ml of *water,* adjusted to pH 6.1 with *concentrated ammonia,* 17 volumes of *acetonitrile* and 8 volumes of *methanol,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Name	Relative retention time
Lincomycin impurity C[1]	0.4
Lincomycin B	0.5
Lincomycin impurity A[2]	0.7
Lincomycin (Retention time: about 10 minutes)	1.0
Lincomycin impurity B[3]	1.2 and 1.3

[1]methyl6,8-dideoxy-6-[[[(2*S*,4*R*)-4-propylpyrrolidin-2-yl] carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (*N*-desmethyl *lincomycin*),

[2]methyl6,8-dideoxy-6-[[[(2*R*,4*R*)-1-methyl-4-propylpyrrolidin-2-yl] carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (α-amide epimer),

[3]methyl 6,8-dideoxy-6-[[[(2*S*,4*EZ*)-1-methyl-4-propylidenepyrrolidin-2-yl]carbonyl]amino]-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (propylidene analogues).

Inject reference solution (c). The test is not valid unless the resolution between the peak due to lincomycin and 1st peak of lincomycin impurity B is not less than 1.8.

Inject reference solution (b) and the test solution. Run the chromatogram 5.5 times the retention time of the principal peak for the test solution. The area of any peak corresponding to lincomycin impurity A is not more than 0.5 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any peak corresponding to lincomycin impurity C is not more than 0.2 times the area of principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the sum of areas of the peaks due to impurity B is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). 3.1 per cent to 4.6 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{35}ClN_2O_6S$ (lincomycin) and $C_{17}H_{33}ClN_2O_6S$ (lincomycin B).

Lincomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unitper mg.

Lincomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Lincomycin Capsules

Lincomycin Hydrochloride Capsules

Lincomycin Capsules contain Lincomycin Hydrochloride equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lincomycin, $C_{18}H_{34}N_2O_6S$.

Usual strengths. 250 mg, 500 mg.

Identification

A. Extract a quantity of the capsules contents containing the equivalent of 0.2 g of lincomycin with a mixture of 4 volumes of *chloroform* and 1 volume of *methanol*, filter and evaporate the filtrate. Dissolve the oily residue in 1 ml of *water*, add *acetone* until precipitation begins and further add 20 ml of *acetone*. Filter the precipitate, wash with two 10 ml quantities of *acetone*, dissolve the residue in a little of the chloroform–methanol mixture (4:1), evaporate to dryness and dry at 60° at a pressure not exceeding 2 kPa for 4 hours. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lincomycin hydrochloride IPRS* or with the reference spectrum of lincomycin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Lincomycin B. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Inject the test solution. The area of any peak corresponding to lincomycin B is not more than 5 per cent of the area of the peak due to lincomycin, calculated by area normalisation method.

Water (2.3.43). Not more than 7.0 per cent, determined on 0.3 g of the contents of capsules.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the content of 20 capsules. Disperse a quantity of the mixed contents containing 50 mg of lincomycin in 50.0 ml of the mobile phase, shake mechanically for a minimum of 5 minutes and filter.

Reference solution. A 0.12 per cent w/v solution of *lincomycin hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 78 volumes of a buffer solution prepared by dissolving 13.5 ml of *orthophosphoric acid* in 1000 ml of *water*, adjusted to pH 6.0 with *ammonium hydroxide*, 15 volumes of *acetonitrile* and 15 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

The relative retention time with reference to lincomycin for lincomycin B is about 0.5.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 1.3 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

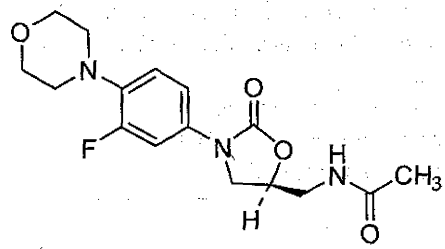
Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{34}N_2O_6S$ in the capsules.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of lincomycin.

Linezolid



$C_{16}H_{20}FN_3O_4$ Mol. Wt. 337.4

Linezolid is *N*-[[(5*S*)-3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide.

Linezolid contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{20}FN_3O_4$, calculated on the dried basis.

Category. Antibacterial.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *linezolid IPRS* or with the reference spectrum of linezolid.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Specific optical rotation (2.4.22). – 14.0° to – 9.0°, determined on 0.9 per cent w/v solution in *chloroform*.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. A solution prepared by diluting 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*.

Solvent mixture. 60 volumes of buffer solution and 40 volumes of *methanol*.

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *linezolid IPRS* in the solvent mixture.

Reference solution (b). Dilute 2 ml of reference solution (a) to 100 ml with the solvent mixture. Further dilute 5.0 ml of the solution to 20.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature: 40°,
- mobile phase: A. a mixture 90 volumes of buffer solution and 10 volumes of *methanol*,
  B. a mixture 10 volumes of buffer solution and 90 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
40	70	30
50	20	80
57	20	80
60	90	10
65	90	10

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Heavy Metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. A solution prepared by diluting 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*.

Solvent mixture. 60 volumes of buffer solution and 40 volumes of *methanol*.

Test solution. Dissolve about 100 mg of the substance under examination in 100.0 ml of the solvent mixture. Dilute 10.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *linezolid IPRS* in the solvent mixture.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature: 40°,
- mobile phase: a mixture of 78 volumes of a solution containing 90 volumes of buffer solution and 10 volumes of *methanol* and 22 volumes of a solution containing 10 volumes of buffer solution and 90 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 2000, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate content of $C_{16}H_{20}FN_3O_4$.

Storage. Store protected from light and moisture, at a temperature not exceeeding 30°.

Linezolid Tablets

Linezolid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of linezolid, $C_{16}H_{20}FN_3O_4$.

Usual strength. 600 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 0.9 g of *sodium hydroxide* in 1000 ml of *water*, adjusted to pH 6.8 with *sodium hydroxide solution*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{16}H_{20}FN_3O_4$ in the medium from the absorbance obtained from a solution of known concentration of *linezolid IPRS* in the same medium.

Q. Not less than 70 per cent of the stated amount of $C_{16}H_{20}FN_3O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of buffer solution prepared by diluting 1.0 ml of *triethylamine* to 1000.0 ml with *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 25 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 40 mg of linezolid with 35 ml of the solvent mixture, sonicate for 20 minutes and dilute to 50.0 ml with the solvent mixture, filter.

Reference solution. A 0.0008 per cent w/v solution of *linezolid IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature: 40°,
- mobile phase: A. a mixture of 90 volumes of buffer solution prepared by mixing 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 10 volumes of *methanol*,

B. a mixture of 10 volumes of buffer solution prepared by mixing 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 90 volumes of *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile Phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0	90	10
35	65	35
45	20	80
47	20	80
48	90	10
55	90	10

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not more than two times the area of the peak in the chromatogram obtained with the reference solution (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 75 volumes of buffer solution prepared by diluting 1.0 ml of *triethylamine* to 1000 ml with *water*, adjusted pH 3.0 with *orthophosphoric acid* and 25 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 40 mg of Linezolid with 35 ml of solvent mixture, sonicate for 20 minutes and dilute to 50 ml with the solvent mixture, filter. Dilute 5 ml of the solution to 50 ml with the solvent mixture.

Reference solution. A 0.08 per cent w/v solution of *linezolid IPRS* in the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature: 40°,
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by diluting 1.0 ml of *triethylamine* to 1000 ml with *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 30 volumes of *methanol*,

- flow rate: 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent

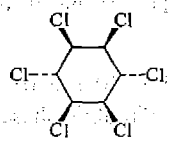
Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{20}FN_3O_4$ in the tablets.

Storage. Store protected from light and moisture.

Lindane

Gamma Benzene Hexachloride



$C_6H_6Cl_6$ Mol.Wt.290.8

Lindane is 1α,2α,3β,4α,5α,6β-hexachlorocyclohexane.

Lindane contains not less 99.0 per cent and not more than 100.5 per cent of $C_6H_6Cl_6$.

Category. Topical parasiticide.

Description. A white or almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lindane IPRS* or with the reference spectrum of lindane.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve about 5 mg in 4 ml of *ethanol (95 per cent)*. Add 1 ml of *0.5 M ethanolic potassium hydroxide* and allow to stand for 10 minutes. The solution gives reaction (A) of chlorides (2.3.1).

D. Melts at 112° to 115° (2.4.21).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *acetone* is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *cyclohexane* and 10 volumes of *chloroform*.

Test solution (a). Dissolve 1 g of the substance under examination in 10 ml of *chloroform*.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *chloroform*.

Reference solution (a). A 1 per cent w/v solution of *lindane IPRS* in *chloroform*.

Reference solution (b). Dilute 1.0 ml of test solution (b) to 10.0 ml with *chloroform*.

Reference solution (c). Dissolve 10 mg of *α-hexachlorocyclohexane IPRS* in sufficient of the test solution (a) to produce 5 ml.

Apply to the plate 1 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of dry air and irradiate with ultraviolet light at 254 nm for 15 minutes. Spray with a 0.6 per cent w/v solution of *dicarboxidine hydrochloride* in *ethanol (90 per cent)* and examine the spots in daylight. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides (2.3.12). To 3.75 g, finely powdered, add 15 ml of *water* and boil for 1 minute. Allow to cool, shaking frequently and filter. To 10 ml of the filtrate add 3 ml of *water* and 2 ml of *ethanol (95 per cent)*. The solution complies with the limit test for chlorides (100 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh 0.2 g, add 10 ml of *ethanol (95 per cent)* and warm on a water-bath until dissolved. Cool, add 20 ml of *0.5 M ethanolic potassium hydroxide* and allow to stand for 10 minutes, swirling frequently. Add 50 ml of *water*, 20 ml of *2 M nitric acid*, 25 ml of *0.1 M silver nitrate* and 5 ml of *ferric ammonium sulphate solution*. Titrate with *0.1 M ammonium thiocyanate* until a reddish-yellow colour is obtained. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of silver nitrate required.

1 ml of *0.1 M silver nitrate* is equivalent to 0.009649 g of $C_6H_6Cl_6$.

Storage. Store protected from light.

Absorbent Lint

Lint; Cotton Lint; Unmedicated Lint

Absorbent Lint is a cotton cloth of plain weave, on one side of which a nep has been raised from either warp or weft yarns. It absorbs water readily but its absorbency may be

considerably reduced by medication, the absorbency of the product depending upon the medicament incorporated.

Category. Surgical dressing.

Description. Cotton cloth of plain weave, reasonably free from weaving defects, readily tearable in both directions and bleached to a good white having on one side a nep raised from either the warp or weft yarns and reasonably free from neps; it is clean and reasonably free from leaf, shell and other foreign substances. It is made of yarn that is reasonably free from slubs, snarls and other defects.

Absorbent Lint has not less than 98.0 per cent of the dimensions stated on the label.

Tests

Threads per cm. Warp not less than 16 and weft not less than 10.

Weight per unit area. 25 g has a superficial area of 1350 to 1370 sq. cm.

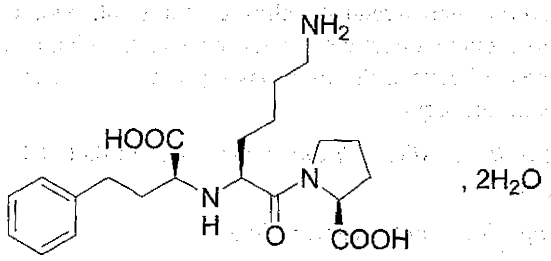
Absorbency. A piece 10 cm square, placed lightly by means of forceps, unraised side downwards, on the surface of water at 20°, becomes saturated within 10 seconds.

Fluorescence. Not more than a few points of fluorescence are visible under screened ultraviolet light.

Storage. Store protected from moisture in well-closed packages in a dry place, free from dust.

Labelling. The label states the dimensions viz. the length and width in cm.

Lisinopril



$C_{21}H_{31}N_3O_5, 2H_2O$ Mol. Wt. 441.5

Lisinopril is (*S*)-1-[*N*^{2}-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate.

Lisinopril contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{31}N_3O_5$, calculated on the anhydrous basis.

Category. Antihypertensive.

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lisinopril IPRS* or with the reference spectrum of lisinopril.

B. In the Assay, the chromatogram obtained with the test solution corresponds to the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). –123.0° to –115.0°, determined on 1.0 per cent w/v solution in *0.25 M zinc acetate* at 405 nm.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 10.0 ml of mobile phase A.

Reference solution (a). A 0.2 per cent w/v solution of *lisinopril IPRS* in mobile phase A.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (Such as Symmetry C8),
- column temperature: 50°,
- mobile phase: A. a mixture of 3 volumes of *acetonitrile* and 97 volumes of *0.02 M sodium dihydrogen phosphate*, adjusted to pH 5.0 with a 5 per cent w/v solution of *sodium hydroxide* and filter,
  B. a mixture of 20 volumes of *acetonitrile* and 80 volumes of *0.02 M sodium dihydrogen phosphate*, adjusted to pH 5.0 with 5 per cent w/v solution of *sodium hydroxide* and filter,
- a gradient programme using the conditions given below,
- flow rate: 1.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
35	70	30
45	70	30
55	0	100
65	100	0
75	100	0

Inject reference solution (b). Test is not valid unless the tailing factor is not more than 3.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 8.0 to 9.5, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30 mg of the substance under examination in 100.0 ml of *water* and filter.

Reference solution. A 0.03 per cent w/v solution of *lisinopril IPRS* in *water.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with dimethyloctylsilane (C8 alkyl chain) fully endcapped stationary phase (5 mm) (Such as Hypersil MOS),
- column temperature: 50°,
- mobile phase: a mixture of 96 volumes of buffer pH 5.0 prepared by dissolving 2.76 g of *monobasic sodium phosphate* in 1000 ml of *water*, adjusted to pH 5.0 with *1 M sodium hydroxide* and 4 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 180 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{31}N_3O_5$.

Storage. Store protected from moisture, at a temperature not exceeding 25°.

Lisinopril Tablets

Lisinopril Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lisinopril, $C_{21}H_{31}N_3O_5$.

Usual strengths. 2.5 mg; 5 mg; 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. Dissolve an accurately weighed quantity of *lisinopril IPRS* in dissolution medium and dilute with dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (Such as Symmetry C8),
- column temperature: 50°,
- mobile phase: dissolve 1.0 g of *hexane sulphonic acid sodium salt* in 800 volumes of *phosphate solution* prepared by dissolving 4.1 g of *monobasic potassium phosphate* in 900 ml *water*, adjusted to pH 2.0 with *orthophosphoric acid,* dilute to 1000 ml with *water* and 200 volumes of *acetonitrile*, mix, filter and degas.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 850 theoretical plates, the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

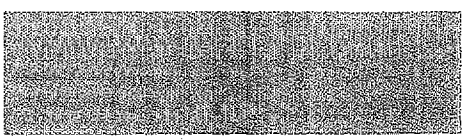
Inject the reference solution and the test solution.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{31}N_3O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a powdered tablet containing 100 mg of Lisinopril to a 50-ml volumetric flask. Add about 25 ml of mobile phase A and sonicate for 10 minutes with intermittent shaking. Make up the volume with the mobile phase A and filter.

Reference solution (a). A 0.2 per cent w/v solution of *lisinopril IPRS* in the mobile phase A.



Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with packed with dimethyloctylsilane (C8 alkyl chain) fully endcapped stationary phase (5 µm) (Such as Hypersil MOS/ Symmetry C8),
- column temperature: 50°,
- mbile phase: A. a mixture of 3 volumes of *acetonitrile* and 97 volumes of *0.02 M sodium dihydrogen phosphate*, adjusted to pH 5.0 with 5 per cent w/v solution of *sodium hydroxide* and filter,
  B. a mixture of 20 volumes of *acetonitrile* and 80 volumes of *0.02 M sodium dihydrogen phosphate*, adjusted to pH 5.0 with 5 per cent w/v solution of *sodium hydroxide* and filter,
- a gradient programme using the conditions given below,
- flow rate: 1.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
35	70	30
45	70	30
50	100	0
60	100	0

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 4 volumes of *water* and 1 volume of *methanol*.

Test solution. Transfer to a suitable size volumertric flask 10 tablets, add the solvent mixture to fill about half of the volumetric flask, shake the flask by mechanical means for 20 minutes and dilute with solvent mixture which will yield a solution having a concentration of about 0.02 per cent w/v and filter.

Reference solution. A 0.02 per cent w/v solution of *lisinopril IPRS* in solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm), (Such as Symmetry C8),
- column temperature: 50°,
- mobile phase: dissolve 1.0 g of *hexane sulphonic acid sodium salt* in 800 volumes of *phosphate solution* prepared by dissolving 4.1 g of *monobasic potassium phosphate* in 900 ml *water*, adjusted to pH 2.0 with *orthophosphoric acid*, dilute to 1000 ml with *water* and 200 volumes of *acetonitrile*, mix, filter and degas.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 850 theoretical plates, the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{31}N_3O_5$.

Storage. Store protect from moisture, at a temperature between 20° to 25°.

Lithium Carbonate

Li_2CO_3 Mol.Wt.73.9

Lithium Carbonate contains not less than 98.5 per cent and not more than 100.5 per cent of Li_2CO_3.

Category. Antidepressant.

Description. A white, crystalline powder.

Identification

A. When moistened with *hydrochloric acid* and introduced on a platinum wire, it imparts a red colour to a non-luminous flame.

B. Dissolve 0.2 g in 1 ml of *hydrochloric acid* and evaporate to dryness on a water-bath; the residue is soluble in 3 ml of *ethanol (95 per cent)*.

C. It gives reaction (A) of carbonates (2.3.1).

Tests

Appearance of solution. Suspend 10.0 g in 30 ml of *distilled water* and dissolve by adding 22 ml of *nitric acid*. Neutralise with *2 M sodium hydroxide* and dilute to 100.0 ml with *distilled water* (solution A). The solution is clear (2.4.1) and colourless (2.4.1).

Arsenic (2.3.10). Dissolve 5.0 g in 15 ml of *brominated hydrochloric acid*, add 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Calcium and magnesium. Dissolve 1.0 g in 30 ml of *1 M hydrochloric acid* and neutralise with *dilute ammonia solution*, filter, if necessary and divide into two portions; to one portion add 1 ml of *ammonium oxalate solution*; no turbidity or precipitate is produced on standing for 5 minutes. To the other portion add 1 ml of *disodium hydrogen phosphate solution*; no turbidity or precipitate is produced on standing for 5 minutes.

Heavy metals (2.3.13). Mix 1.0 g with 5 ml of *water* and 15 ml of *dilute hydrochloric acid*, heat to boiling and maintain that temperature for 1 minute. Add 1 drop of *phenolphthalein solution* and sufficient *ammonia solution* to give the solution a faint pink colour. Cool and dilute to 25 ml with *water*. The resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

Iron (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).

Potassium. Dissolve 1.0 g in 10 ml of *7 M hydrochloric acid*, add sufficient *water* to produce 50 ml and determine by Method A of flame photometry (2.4.4) or by Method A of Atomic absorption spectrophotometry (2.4.2), measuring at 767 nm, using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water*, to prepare the standard solution (500 ppm).

Sodium. Dissolve 1.0 g in 10 ml of *7 M hydrochloric acid*, add sufficient *water* to produce 50 ml and determine by Method A for flame photometry (2.4.4) or by Method A of Atomic absorption spectrophotometry (2.4.2), measuring at 589 nm, using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water*, to prepare the standard solution (500 ppm).

Chlorides (2.3.12). 10 ml of solution A diluted to 15 ml with *water* complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). Disperse 0.75 g in 5 ml of *distilled water* and dissolve by adding 5 ml of *7 M hydrochloric acid*. Boil for 2 minutes, cool, neutralise with *2 M sodium hydroxide* and dilute to 25 ml with *distilled water*. The resulting solution complies with the limit test for sulphates (200 ppm).

Assay. Dissolve 0.5 g in 25.0 ml of *1 M hydrochloric acid* and titrate with *1 M sodium hydroxide* using *methyl orange solution* as indicator.

Repeat the operation without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of *1 M hydrochloric acid* is equivalent to 0.03695 g of Li_2CO_3.

Storage. Store protected from moisture.

Lithium Carbonate Prolonged-release Tablets

Lithium Carbonate Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Lithium Carbonate Prolonged-release Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of content of lithium carbonate, Li_2CO_3.

Usual strengths. 300 mg; 400 mg; 450 mg.

Identification

A small quantity of the powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a red colour to the flame.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 1 g of Lithium Carbonate in 100 ml of *water* and 50 ml of *1 M hydrochloric acid* and boil for 1 minute to remove carbon dioxide. Cool and titrate with *1 M sodium hydroxide* using *methyl orange solution* as indicator. Carry out a blank titration.

1 ml of *1 M hydrochloric acid* is equivalent to 0.03695 g of Li_2CO_3.

Lithium Carbonate Tablets

Lithium Carbonate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of lithium carbonate, Li_2CO_3.

Usual strength. 300 mg.

Identification

A small quantity of the powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire, imparts a red colour to a non-luminous flame.

Tests

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 30 minutes.

Withdraw 90.0 ml of the medium, add a drop of *hydrochloric acid* and dilute to 100.0 ml with *water*. Filter and dilute with *water*, if necessary. Determine by flame photometry Method A (2.4.4), or by atomic absorption spectrophotometry, Method A (2.4.2), measuring at 671 nm and using *lithium solution FP*, or *lithium solution AAS*, as appropriate, suitably diluted with *water*, for the standard solution.

Calculate the content of Li_2CO_3 in the medium.

Q. Not less than 60 per cent of the stated amount of Li_2CO_3.

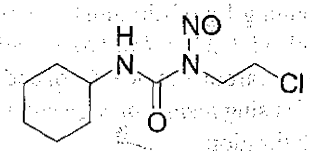
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 1 g of Lithium Carbonate, add 100 ml of *water* and 50.0 ml of *1 M hydrochloric acid* and boil for 1 minute to remove carbon dioxide. Cool and titrate with *1 M sodium hydroxide* using *methyl orange solution* as indicator. Carry out a blank titration.

1 ml of *1 M hydrochloric acid* is equivalent to 0.03695 g of Li_2CO_3.

Storage. Store protected from moisture.

Lomustine



$C_9H_{16}ClN_3O_2$ Mol. Wt. 233.7

Lomustine is 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

Lomustine contains not less than 98.5 per cent and not more than 100.5 per cent of $C_9H_{16}ClN_3O_2$, calculated on the dried basis.

Category. Anticancer.

Description. A yellow, crystalline powder.

Carry out the tests protected from light and prepare the solutions immediately before use.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lomustine IPRS* or with the reference spectrum of lomustine.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 230 nm, about 0.52.

C. In the test A for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. Dissolve about 25 mg in 1 ml of *methanol*, add 0.1 ml of *2 M sodium hydroxide* and 2 ml of *water* and acidify by adding, dropwise, *1 M nitric acid*. The resulting solution gives the reactions of chlorides (2.3.1).

Tests

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid*.

Test solution (a). Dissolve 0.25 g of the substance under examination in 10.0 ml of *methanol*.

Test solution (b). Dissolve 0.1 g of the substance under examination in 100.0 ml of *methanol*.

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (b). A 0.005 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (c). A 0.1 per cent w/v solution of *lomustine IPRS* in *methanol*.

Reference solution (d). A solution containing 0.1 per cent w/v each of *lomustine IPRS* and *1,3-dicyclohexylurea* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 110° for 1 hour, exposing the hot plate in a closed tank containing chlorine, produced by adding *dilute hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed at the bottom of tank. Allow the plate to stand in contact with the chlorine vapours for 5 minutes. Remove the plate from the tank and dry it in a current of cold air until the excess of chlorine is removed and an area of the plate below the line of application produces at most a very faint blue colour with 0.05 ml of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.

B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.25 g of the substance under examination in 10 ml of *methanol*.

Reference solution. A 0.025 per cent w/v solution of the substance under examination in *methanol*.

Chromatographic system
- a stainless steel column 20 cm × 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: equal volumes of *methanol* and *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The retention time of lomustine is about 25 minutes. When using a recorder, adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is not less than 50 per cent of the full scale of the recorder.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the sum of areas of any secondary peaks is not greater than the area of the peak in the chromatogram obtained with the reference solution. Ignore any peak with an area less than one-twentieth of that of the principal peak in the chromatogram obtained with the reference solution.

Chlorides (2.3.12). Dissolve 0.24 g in 4 ml of *methanol*, add 20 ml of *water*, allow to stand for 20 minutes and filter. To 10 ml of the filtrate add 5 ml of *methanol*. The resulting solution complies with the limit test for chlorides, replacing the 5 ml of *water* in the standard solution with 5 ml of *methanol (0.25 per cent)*.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

Assay. Weigh 0.2 g, add 20 ml of a 20 per cent w/v solution of *potassium hydroxide* and boil under a reflux condenser for 2 hours. Add 75 ml of *water* and 4 ml of *nitric acid*, cool. Titrate with *0.05 M silver nitrate*, determining the end-point potentiometrically (2.4.25). Repeat the operation without the substance under examination. The difference between the titrations represents the amount of *silver nitrate* required.

1 ml of *0.05 M silver nitrate* is equivalent to 0.01168 g of $C_9H_{16}ClN_3O_2$.

Storage. Store protected from light and moisture.

Lomustine Capsules

Lomustine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lomustine, $C_9H_{16}ClN_3O_2$.

Usual strengths. 10 mg; 40 mg.

Carry out the tests protected from light and prepare the solutions immediately before use.

Identification

Shake a quantity of the contents of the capsules containing 0.2 g of Lomustine with 10 ml of *methanol*, filter and evaporate the filtrate to dryness using a rotary evaporator on a water-bath maintained at not more than 60°. The residue, after drying at a pressure not exceeding 0.7 kPa at 60° for 30 minutes, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lomustine IPRS* or with the reference spectrum of lomustine.

B. Melting range. 89° to 91° (2.4.21).

Tests

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *toluene* and 20 volumes of *glacial acetic acid*.

Test solution (a). Shake a quantity of the contents of the capsules containing 0.25 g of Lomustine with 10 ml of *methanol* and filter.

Test solution (b). Dilute 1 volume of test solution (a) to 250 volumes with *methanol*.

Reference solution (a). Dilute 1 volume of test solution (b) to 2 volumes with *methanol*.

Reference solution (b). A 0.005 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (c). A 0.1 per cent w/v solution of *lomustine IPRS* in *methanol*.

Reference solution (d). A solution containing 0.1 per cent w/v each of *lomustine IPRS* and *1,3-dicyclohexylurea* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate, heat it at 110° for 1 hour, exposing the hot plate in a closed tank containing chlorine, produced by adding *dilute hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed at the bottom of tank. Allow the plate to stand in contact with the chlorine vapours for 5 minutes. Remove the plate and dry it in a current of cold air until the excess of chlorine is removed and an area of the plate below the line of application produces at most a very faint blue colour with 0.05 ml of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution*. Any secondary spot in the chromatogram obtained with test

solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b). The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated principal spots.

B. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the contents of the capsules containing 0.25 g of Lomustine with 10 ml of *methanol* and filter.

Reference solution. Dilute 1.0 ml of the test solution to 50.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 20 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 mm),
- mobile phase: equal volumes of *methanol* and *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The retention time of lomustine is about 25 minutes. When using a recorder, adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with the reference solution is not less than 50 per cent of the full scale of the recorder.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the sum of areas of any secondary peaks is not greater than the area of the peak in the chromatogram obtained with the reference solution. Ignore any peak with an area less than one-twentieth of that of the principal peak in the chromatogram obtained with the reference solution.

Uniformity of content. Complies with the test stated under Capsules.

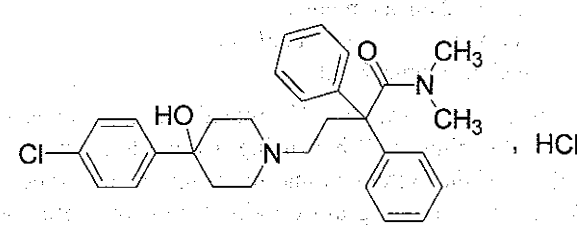
Transfer the contents of a capsule quantitatively to a 100 ml volumetric flask with the aid of 20 ml of *ethanol (95 per cent)*, shake well, make up to the volume with *ethanol (95 per cent)* and filter. Dilute suitably with the same solvent and measure the absorbance of the resulting solution at the maximum at about 230 nm (2.4.7). Calculate the content of $C_9H_{16}ClN_3O_2$ in the capsule taking 260 as the specific absorbance at 230 nm.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 40 mg of Lomustine and shake with 70 ml of *ethanol (95 per cent)* for 20 minutes, dilute to 100.0 ml with *ethanol (95 per cent)* and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 230 nm (2.4.7). Calculate the content of $C_9H_{16}ClN_3O_2$ taking 260 as the specific absorbance at 230 nm.

Storage. Store protected from light and moisture.

Loperamide Hydrochloride



$C_{29}H_{33}ClN_2O_2,HCl$ Mol. Wt. 513.5

Loperamide Hydrochloride is 4-(4-chlorophenyl)-4-hydroxy-piperidino]-*N*,*N*-dimethyl-2,2-diphenylbutyramide hydrochloride.

Loperamide Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{29}H_{33}ClN_2O_2,HCl$, calculated on the dried basis.

Category. Antidiarrhoeal.

Description. A white or almost white powder.

Identification

Test B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *loperamide hydrochloride IPRS* or with the reference spectrum of loperamide hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *octadecylsilyl silica gel*.

Mobile phase. A mixture of 40 volumes of *dioxan*, 40 volumes of *methanol* and 20 volumes of *ammonium acetate*.

Test solution. Dissolve 0.6 g of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). A 0.6 per cent w/v solution of *loperamide hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.6 per cent w/v each of *loperamide hydrochloride IPRS* and *ketoconazole IPRS* in the mobile phase.

Apply to the plate 5 µl of each solution. After development, dry the plate in air for 15 minutes and expose it to iodine vapours until the spots appear. The principal spot in the

chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Dissolve 50 mg in a mixture of 0.4 ml of *strong ammonia solution* and 2 ml of *water*. Mix, allow to stand for 5 minutes and filter. Acidify the filtrate with *2 M nitric acid*. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

Reference solution (a). A solution containing 0.0025 per cent w/v each of *loperamide hydrochloride IPRS* and *haloperidol IPRS* in *methanol*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 20.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 1.7 per cent w/v solution of *tetrabutylammoniumhydrogen sulphate*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
10	30	70
15	30	70
20	90	10

Equilibrate the column for at least 30 minutes with *acetonitrile* and then equilibrate at the initial eluent composition for at least 5 minutes.

Adjust the sensitivity of the detector so that the height of the principal peak in the chromatogram obtained with reference solution (b) is 70 per cent to 90 per cent of the full scale of the recorder.

The retention times are: haloperidol, about 3 minutes and loperamide hydrochloride, about 4.5 minutes.

Inject reference solution (a). The test is not valid unless the resolution between the peaks corresponding to haloperidol and loperamide hydrochloride is at least 8.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent) and the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.4 g, dissolve in 25 ml of anhydrous *glacial acetic acid*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *alpha-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05135 g of $C_{29}H_{33}ClN_2O_2,HCl$.

Storage. Store protected from light and moisture.

Loperamide Capsules

Loperamide Hydrochloride Capsules

Loperamide Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of loperamide hydrochloride $C_{29}H_{33}ClN_2O_2,HCl$.

Usual strength. 2 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 85 volumes of *chloroform*, 10 volumes of *methanol* and 5 volumes of *formic acid*.

Test solution. To a quantity of the contents of the capsules, containing about 10 mg of Loperamide Hydrochloride, add 10 ml of *methanol*, shake for 5 minutes and filter.

Reference solution. A 0.1 per cent w/v solution of *loperamide hydrochloride IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapour. The principal spot in the chromatogram obtained with the test solution

corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2)

Apparatus No. 1 (Basket),
Medium. 500 ml of pH 4.7 acetate buffer, prepared by mixing 200 ml of *1 M acetic acid* with 600 ml of *water*, adjusting with *1 M sodium hydroxide* to a pH of 4.70 ± 0.05, diluting with *water* to 1000 ml and mixing,
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution of *loperamide hydrochloride IPRS* in the dissolution medium containing the same concentration of loperamide hydrochloride as that expected in the dissolution medium in the vessel.

Determine by liquid chromatography (2.4.14) using the chromatographic system described under Assay.

Calculate the content of $C_{29}H_{33}ClN_2O_2$,HCl in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{29}H_{33}ClN_2O_2$, HCl.

Uniformity of content. Complies with the test stated under Capsules.

Transfer the contents of one capsule to a 200-ml volumetric flask. Add 35 ml of *0.5 M hydrochloric acid* and mix with the aid of ultrasound for 15 minutes. Add 35 ml of *acetonitrile* and mix with the aid of ultrasound for another 15 minutes. Dilute to volume with a mixture of equal volumes of *0.5 M hydrochloric acid* and *acetonitrile* mix and filter.

Determine by liquid chromatography (2.4.14) using the chromatographic system and the reference solution described under Assay.

Calculate the content of $C_{29}H_{33}ClN_2O_2$, HCl in the capsule.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately weighed portion of the mixed contents of 20 capsules containing about 20 mg of Loperamide Hydrochloride, to a 100 ml volumetric flask. Add about 35 ml of *0.5 M hydrochloric acid* and mix with the aid of ultrasound for 15 minutes. Add 35 ml of *acetonitrile* and mix with the aid of ultrasound for an additional 15 minutes. Dilute with a mixture of equal volumes of *acetonitrile* and *0.5 M hydrochloric acid*, mix and filter. Transfer 5.0 ml of the solution to a 100 ml volumetric flask, dilute to volume with the same solvent mixture and mix.

Reference solution. A 0.001 per cent w/v solution of *loperamide hydrochloride IPRS* in a mixture of equal volumes of *acetonitrile* and *0.5 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 25 cm × 4 mm, packed with nitrile groups chemically bonded to porous silica particles (10 µm),
- mobile phase: dilute 500 ml of *acetonitrile* to 1000.0 ml with *water*, add 20 drops of *phosphoric acid*, mix and filter,
- flow rate: 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

Inject the reference solution. The column efficiency, determined from the analyte peak is not less than 1900 theoretical plates, the capacity factor, is not less than 3.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{29}H_{33}ClN_2O_2$,HCl in the capsules.

Loperamide Tablets

Loperamide Hydrochloride Tablets

Loperamide Hydrochloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of loperamide hydrochloride $C_{29}H_{33}ClN_2O_2$,HCl.

Usual strength. 2 mg.

Identification

A. Disperse a quantity of powdered tablets containing about 10 mg of Loperamide Hydrochloride, to a test-tube, add 20.0 ml of *isopropyl alcohol*. shake by mechanical means for one minute and allow to settle. Dilute 9.0 ml of the supernatant to 10 ml with *0.1M hydrochloric acid*. The solution so obtained, when examined in the range of 250 nm to 300 nm (2.4.7) shows absorption maxima and minima at the same wavelengths as that of a similar preparation of *loperamide hydrochloride IPRS*.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Test Solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution of *loperamide hydrochloride IPRS* in the dissolution medium containing the same concentration of loperamide hydrochloride as that expected in the dissolution medium in the vessel.

Determine by liquid chromatography (2.4.14) using the chromatographic system described under Assay.

Calculate the content of $C_{29}H_{33}ClN_2O_2$, HCl in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{29}H_{33}ClN_2O_2$, HCl.

Uniformity of content. Complies with the test stated under Tablets.

Test solution. Disperse 1 tablet to a 200-ml volumetric flask. Add 4 ml of a 5 per cent w/v solution of *phosphoric acid* and 20 ml of *methanol* shake and dilute to volume with *water*.

Reference solution. Dissolve an accurately weighed quantity of *loperamide hydrochloride IPRS* in *methanol* to obtain a solution having a known concentration of about 2 mg per ml. Quantitatively dilute the solution with *water* to obtain a solution having a known concentration of about 0.2 mg per ml. To 10.0 ml of the solution add 4.0 ml of 5 per cent *phosphoric acid solution* and 20 ml of *methanol*, dilute with *water* to 200.0 ml and mix.

Determine by liquid chromatography (2.4.14) using the chromatographic system described under Assay.

Calculate the content of $C_{29}H_{33}ClN_2O_2$, HCl in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution. To 3.0 g of *triethylamine hydrochloride* and 1.0 ml of *phosphoric acid* add 550 ml of *water* and mix.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 16 mg of Loperamide Hydrochloride, to a 200-ml volumetric flask. Add 4 ml of a 5 per cent w/v solution of *phosphoric acid* and 20 ml of *methanol*, dilute with *water* to volume and mix. To 10.0 ml of the solution, add 2.0 ml of 5 per cent w/v solution of *orthophosphoric acid* and 10 ml of *methanol* and dilute to 100.0 ml with *water*.

Reference solution. Dissolve an accurately weighed quantity of *loperamide hydrochloride IPRS* in *methanol* to obtain a solution having a known concentration of about 2 mg per ml. Quantitatively dilute this solution with *water* to obtain a solution having a known concentration of about 0.2 mg per ml. Transfer 10.0 ml of the solution to a 250-ml volumetric flask, add 5.0 ml of 5 per cent *phosphoric acid solution* and 25 ml of *methanol*, dilute with *water* to volume and mix.

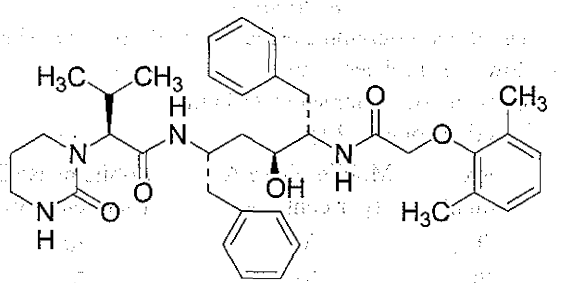
Chromatographic system
- a stainless steel column 8 cm x 4 mm, packed with octylsilane chemically bonded to totally porous silica particles (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of buffer solution,
- flow rate: 2 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 20 µl.

Inject the reference solution. The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{29}H_{33}ClN_2O_2$,HCl in the tablets.

Lopinavir



$C_{37}H_{48}N_4O_5$ Mol. Wt. 628.8

Lopinavir is (αS)-*N*-[(1*S*,3*S*,4*S*)-4-[[(2,6-dimethylphenoxy)acetyl]amino-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]tetrahydro-α-(1-methylethyl)-2-oxo-1-(2*H*)-pyrimidineacetamide.

Lopinavir contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{37}H_{48}N_4O_5$, calculated on the anhydrous basis.

Category. Antiretroviral.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lopinavir IPRS* or with the reference spectrum of lopinavir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to lopinavir in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). –26.0° to –22.0°, determined in a 0.4 per cent w/v solution in *methanol* and calculated on the anhydrous basis.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH 2.5 with *phosphoric acid* and diluting to 1000 ml with *water* and 30 volumes of *acetonitrile*.

Test solution. Dissolve 15 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution. A 0.015 per cent w/v solution of *lopinavir IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. buffer solution pH 2.5,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
30	30	70
40	20	80
50	20	80

Inject the reference solution. The test is not valid unless the column efficiency determined from the lopinavir peak is not less than 5000 theoretical plates and the tailing factor is not more than 1.5.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.3 times the area of the peak in the chromatogram obtained with the reference solution (0.3 per cent) and sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* in 900 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and diluting to 1000 ml with *water* and 30 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 10.0 ml of the solution to 50.0 with the solvent mixture.

Reference solution. A 0.02 per cent w/v solution of *lopinavir IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 mm),
- mobile phase: 55 volumes of solution B prepared by mixing 80 volumes of *acetonitrile* and 20 volumes of *methanol* and 45 volumes of *0.05 M potassium dihydrogen phosphate*, adjusted to pH 3.0 with *dilute phosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lopinavir peak is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{37}H_{48}N_4O_5$.

Storage. Store protected from light and moisture.

Lopinavir and Ritonavir Capsules

Lopinavir and Ritonavir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lopinavir, $C_{37}H_{48}N_4O_5$ and ritonavir, $C_{37}H_{48}N_6O_5S_2$.

Usual strength. Lopinavir 200 mg and Ritonavir 50 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a solution prepared by dissolving 15.7 g of *polyoxyethylene 10-lauryl ether* in 1000 ml of a 0.85 per cent v/v solution of *hydrochloric acid*,

Speed and time. 75 rpm and 120 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution containing 0.15 per cent w/v of *lopinavir IPRS* and 0.04 per cent w/v of *ritonavir IPRS* in *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the dissolution medium.

Use the chromatographic system given in the Assay.

Inject the reference solution and the test solution.

Calculate the contents of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$ in the medium.

Q. Not less than 75 per cent of the stated amounts of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$.

Related substances. Determine by liquid chromatography (2.4.14).

For Lopinavir —

Solvent mixture. 40 volumes of *buffer solution* and 60 volumes of *acetonitrile*.

Test solution. Weigh accurately a quantity of the contents of the capsules containing 100 mg of Lopinavir, disperse in 100 ml of the solvent mixture and filter.

Reference solution (a). A 0.1 per cent w/v solution of *lopinavir IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 45 volumes of *acetonitrile* and 55 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate dihydrate* in 1000 ml of *water* and adjusted to pH 4.0 with *orthophosphoric acid*,

  B. a mixture of 80 volumes of *acetonitrile* and 20 volumes of the buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
80	100	0
90	0	100
120	0	100
130	100	0
140	100	0

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

For Ritonavir —

Solvent mixture. 40 volumes of the buffer solution and 60 volumes of *acetonitrile*.

Test solution. Weigh accurately a quantity of the contents of the capsules containing 50 mg of Ritonavir, disperse in 100 ml of the solvent mixture and filter.

Reference solution (a). A 0.05 per cent w/v solution of *ritonavir IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

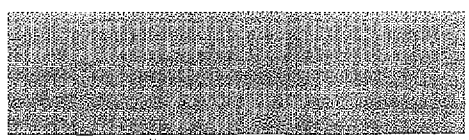
Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with silica gel consisting of porous spherical particles with chemically bonded butyl group (3 µm) (Such as YMC C4),
- column temperature: 60°,
- mobile phase: A. a mixture of 69 volumes of buffer solution prepared by dissolving 4.1 g of *monobasic potassium phosphate* in 1000 ml of *distilled water* and filtering and 18 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n-Butanol*.

  B. a mixture of 40 volumes of the buffer solution, 47 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n-Butanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
60	100	0
120	0	100
130	100	0
155	100	0

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.



Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (b) (5.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 20 capsules. Weigh accurately a quantity of the mixed contents containing 70 mg of Lopinavir and disperse in 100.0 ml of *methanol* and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution. A solution containing 0.070 per cent w/v of *lopinavir IPRS* and 0.0175 per cent w/v of *ritonavir IPRS* in *methanol*. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *buffer solution* prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate dihydrate* in 1000 ml with *water* and adjusted to p*H* 3.0 with *orthophosphoric acid*, 42.5 volumes of *acetonitrile* and 12.5 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the resolution between lopinavir and ritonavir peak is not less than 2.5, the column efficiency for each component is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the contents of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$ in the capsules.

Storage. Store protected from moisture in a refrigerator (2° to 8°).

Lopinavir and Ritonavir Tablets

Lopinavir and Ritonavir Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of lopinavir, $C_{37}H_{48}N_4O_5$ and ritonavir, $C_{37}H_{48}N_6O_5S_2$.

Usual strength. Lopinavir 200 mg and Ritonavir 50 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of a solution prepared by dissolving 15.7 g of *polyoxyethylene 10 lauryl ether* in 1000 ml of a 0.85 per cent v/v solution of *hydrochloric acid*,

Speed and time. 75 rpm and 120 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution containing 0.22 per cent w/v of *lopinavir IPRS* and 0.055 per cent w/v of *ritonavir IPRS* in *methanol*. Dilute 5 ml of the solution to 50 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the contents of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$ in the medium.

Q. Not less than 70 per cent of the stated amounts of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$.

Related substances. Determine by liquid chromatography (2.4.14).

For Lopinavir —

Test solution. Disperse accurately a quantity of the powdered tablets containing 100 mg of Lopinavir in 100 ml of the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *lopinavir IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate in* 1000 ml of *water* and adjusted to pH 4.0 with *orthophosphoric acid* and 45 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,

– spectrophotometer set at 210 nm,
– injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

For Ritonavir —

Solvent mixture. A mixture of 40 volumes of a buffer solution prepared by dissolving 4.1 g of *potassium dihydrogen phosphate* in 1000 ml of *water* and 60 volumes of *acetonitrile*.

Test solution. Disperse an accurately weighed quantity of the powdered tablets containing 100 mg of Ritonavir in 100 ml of the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *ritonavir IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Chromatographic system
– a stainless steel column 15 cm x 4.6 mm, packed with silica gel consisting of porous spherical particles with chemically bonded with butyl group (3 µm) (Such as YMC C4),
– column temperature: 60°,
– mobile phase: A. a mixture of 69 volumes of a buffer solution prepared by dissolving 4.1 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, 18 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n- butanol*,
B. a mixture of 40 volumes of *buffer solution*, 47 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n-butanol*,
– a gradient programme using the conditions given below,
– flow rate: 1 ml per minute,
– spectrophotometer set at 240 nm,
– injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent w/v)
0	100	0
60	100	0
120	0	100
121	100	0
135	100	0

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (b) (5.0 per cent).

Water (2.3.43). Not more than 6.0 per cent, determined on 0.5 g.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 200 mg of Lopinovir in 250.0 ml of *methanol*. Dilute 5.0 ml of the resulting solution to 50.0 ml with the mobile phase.

Reference solution. 5.0 ml of each of a 0.08 per cent w/v solution of *lopinavir IPRS* and a 0.02 per cent w/v solution of *ritonavir IPRS* in *methanol*, diluted to 50.0 ml with the mobile phase.

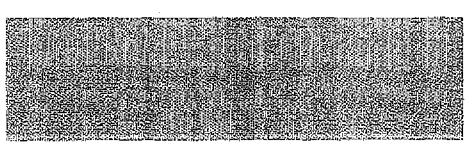
Chromatographic system
– a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
– mobile phase: A. a mixture of 80 volumes of *acetonitrile* and 20 volumes of *methanol*,
B. a mixture of 45 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate anhydrous* in 1000 ml of *water* and adjusted to pH 3.0 with *orthophosphoric acid* and 55 volumes of mobile phase A.
– flow rate: 1.5 ml per minute,
– spectrophotometer set at 210 nm,
– injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peak due to lopinavir (retention time, about 6 minutes) and the peak due to ritonavir (retention time, about 5 minutes) is less than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

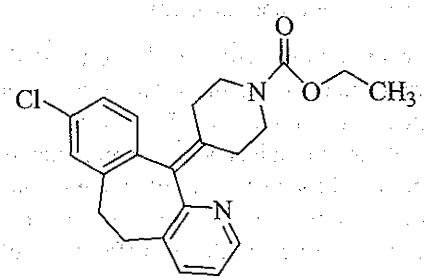
Inject the reference solution and the test solution.

Calculate the contents of $C_{37}H_{48}N_4O_5$ and $C_{37}H_{48}N_6O_5S_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.



Loratadine



$C_{22}H_{23}ClN_2O_2$ Mol. Wt. 382.9

Loratadine is ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene) piperidine-1-carboxylate.

Loratadine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{22}H_{23}ClN_2O_2$, calculated on the dried basis.

Category. H_1 receptor antagonist; antihistamine.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *loratadine IPRS* or with the reference spectrum of loratadine.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

Impurity H. Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.025 per cent v/v solution of *isoamyl benzoate* in *methylene chloride*. Dilute 5.0 ml of the solution to 50.0 ml with same solvent.

Test solution. Dissolve 25 mg of the substance under examination in *methylene chloride* add 1.0 ml of reference solution (a) and 1.0 ml of the internal standard solution and dilute to 5.0 ml with *methylene chloride*.

Reference solution (a). A 0.0025 per cent w/v solution of *loratadine impurity H IPRS* (ethyl 4-oxopiperidine-1-carboxylate) in *methylene chloride*.

Reference solution (b). To 1.0 ml of reference solution (a) add 1.0 ml of the internal standard solution and dilute to 5.0 ml with *methylene chloride*.

Chromatographic system

- a fused silica capillary column 25 m × 0.32 mm, packed with poly(dimethyl) siloxane (film thickness 0.52 µm),
- temperature:

column	time (min.)	temperature (°)
	0	80
	1	80
	23	300
	33	300

- inlet port 260° and detector at 300°,
- split ratio. 1:30,
- flame ionization detector,
- flow rate: 1.0 ml per minute, using helium/nitrogen as the carrier gas,
- injection volume: 1 µl.

Name	Relative retention time
Loratadine Impurity H[1]	0.33
Isoamyl benzoate	0.37
Loratadine (Retention time is about 32 minutes)	1

[1]timeethyl 4-oxopiperidine-1-carboxylate.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to impurity H and isoamyl benzoate is not less than 2.0, the signal to noise ratio is not less than 10 for impurity H peak.

Inject reference solution (b) and the test solution. Calculate the ratio of the area of the peak due to impurity H to the area of the peak due to isoamyl benzoate from the chromatogram obtained with reference solution (b); from the chromatogram obtained with the test solution, calculate the ratio of the area of the peak due to impurity H to the area of the peak due to isoamyl benzoate this ratio is not more than twice (0.1 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution(a). A 0.002 per cent w/v solution of *loratadine impurity F IPRS* in the mobile phase.

Reference solution (b). Dissolve 5 mg of *loratadine for system suitability IPRS* (containing impurity A and impurity E) in the mobile phase, add 0.5 ml of reference solution (a) and dilute to 5.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm),

- column temperature: 40°,
- mobile phase: a mixture of 30 volumes of *methanol*, 35 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 2.8 with *orthophosphoric acid* and 40 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Loratadine impurity D[1]	0.2	—
Loratadine impurity B[2]	0.4	—
Loratadine impurity F[3]	0.9	1.6
Loratadine (Retention time is about 12 minutes)	1.0	—
Loratadine impurity E[4]	1.1	1.9
Loratadine impurity A[5]	2.4	1.7
Loratadine impurity C[6]	2.7	—

[1]8-chloro-11-(piperidin-4-ylidene)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine,

[2]8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine-11-one,

[3]ethyl 4-[(11*RS*)-8-chloro-11-fluoro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]piperidine-1-carboxylate,

[4]ethyl 4-[(11*RS*)-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]-3,6-dihydropyridine-1(2*H*)-carboxylate,

[5]ethyl 4-[(11*RS*)-8-chloro-11-hydroxy-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]piperidine-1-carboxylate,

[6]ethyl 4-(4,8-dichloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine-11-ylidene)piperidine-1-carboxylate.

Inject reference solution (b). The test is not valid unless the peak–to–valley ratio is not less than 2.5, where Hp is the height above the baseline of the peak due to impurity E and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to loratadine.

Inject reference solution (c) and the test solution. Run the chromatogram 5 times the retention times of the principal peak for test solution. In the chromatogram obtained with the test solution, the area of any peak due to impurity F is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent), the area of any peak due to impurity A,B,C,D and E for each impurity are not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent) and the sum of the areas of all secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (c) (0.5 per cent). Ignore any peaks with an area 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (c) (0.05 per cent).

Sulphates (2.3.17). Ignite 1.0 g at 800 ± 25° and take up the residue with 20.0 ml of *distilled water*. Filter, if necessary. The filtrate compiles with the limit test for sulphates (150 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on1.0 g by drying in an oven at 105°.

Assay. Weigh about 0.3 g and dissolve in 50 ml of *anhydrous glacial acetic acid* and titrate with *0.1 M perchloric acid*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03829 g of $C_{22}H_{23}ClN_2O_2$.

Loratadine Tablets

Loratadine Tablets contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{22}H_{23}ClN_2O_2$.

Usual strengths. 5 mg; 10 mg.

Identification

Extract a quantity of the powdered tablets containing 50 mg of loratadine with 20 ml of *acetone*, filter and evaporate the filter to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *loratadine IPRS* or with the reference spectrum of loratadine.

Tests

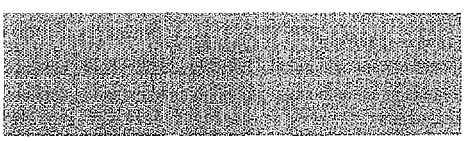
Impurity H. Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.002 per cent w/v solution of *isoamyl benzoate* in *dichloromethane*.

Test solution. Shake a quantity of powdered tablets containing 20 mg of loratadine with 5.0 ml of *dichloromethane* and filter. To the filtrate, add 1.0 ml of the internal standard solution and 1.0 ml of reference solution (a) and dilute to 10.0 ml with *dichloromethane*.

Reference solution (a). A 0.002 per cent w/v solution of *loratadine impurity H IPRS* (ethyl 4-oxopiperidine-1-carboxylate) in *dichloromethane*.

Reference solution (b). To 1.0 ml of internal standard solution add 1.0 ml of reference solution (a) and dilute to 10.0 ml with *dichloromethane*.



Chromatographic system

- a fused silica capillary column 25 m × 0.32 mm, packed with poly(dimethyl) siloxane (film thickness 0.52 µm) (Such as HP1),
- temperature:

column	time (min.)	temperature (°)
	0	80
	1	80
	23	300
	33	300

- inlet port 260° and detector at 300°,
- split ratio. 1:30,
- flame ionization detector,
- flow rate: 1 ml per minute, using helium as the carrier gas,
- injection volume: 1 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to impurity H and isoamyl benzoate is not less than 2.0. Calculate the ratio of the area of the peak due to impurity H to the area of the of the peak due to isoamyl benzoate from the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the ratio of the area of any peak due to impurity H to the area of the peak due to isoamyl benzoate is not more than twice the ratio of the peaks obtained with reference solution (b) (0.1 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Disperse a quantity of powdered tablets containing 20 mg of loratadine with 10.0 ml of *methanol* and filter. Dilute 1.0 ml of the filtrate to 2.0 ml with the mobile phase.

Test solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml and further dilute 1.0 ml to 5.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *loratadine impurity standard IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm) (Such as ODS-3V),
- column temperature: 40°,
- mobile phase: a mixture of 30 volumes of *methanol*, 35 volumes of *0.05 M potassium dihydrogen orthophosphate*, adjusted to pH 2.8 with *orthophosphoric acid* and 40 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Loratadine Impurity F[1]	0.9	1.6
Loratadine Impurity E[2]	1.1	1.9
Loratadine Impurity A[3]	2.4	1.7

[1]ethyl 4-[(11*RS*)-8-chloro-11-fluoro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]piperidine-1-carboxylate,

[2]ethyl 4-[(11*RS*)-8-chloro-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]-3,6-dihydropyridine-1(2*H*)-carboxylate,

[3]ethyl 4-[(11*RS*)-8-chloro-11-hydroxy-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl]piperidine-1-carboxylate.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to impurity E and loratadine is not less than 1.5.

Inject test solution (a) and (b). In the chromatogram obtained with test solution (a), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with test solution (b) (0.2 per cent) and the sum of the areas of all secondary peaks is not more than 2.5 times the area of the peak in the chromatogram obtained with test solution (b) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.05 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions and reference solution (a) as described under Assay.

Test solution. Transfer one tablet in 10-ml volumetric flask, disperse in minimum quantity of *water* and add sufficient quantity of *methanol*, shake for 10 minutes, filter. Dilute the filtrate, with mobile phase to obtain a solution containing 0.01 per cent w/v of loratadine.

Calculate the content of $C_{22}H_{23}ClN_2O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 20 mg of Loratadine with 20.0 ml of *methanol* and filter. Dilute 1.0 ml of the filtrate to 10.0 ml with the mobile phase.

Reference solution(a). A 0.01 per cent w/v solution of *loratadine IPRS* in the mobile phase.

Reference solution (b). A 0.1 per cent w/v solution of *loratadine impurity standard IPRS* in the mobile phase.

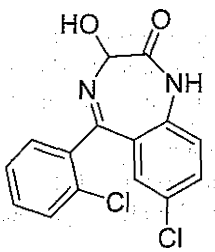
Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to loratadine and impurity E is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{23}ClN_2O_2$ in the tablets.

Lorazepam



$C_{15}H_{10}Cl_2N_2O_2$ Mol. Wt. 321.2

Lorazepam is (3*RS*)-7chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Lorazepam contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{10}Cl_2N_2O_2$, calculated on the dried basis.

Category. Antianxiety.

Description. A white or almost white, crystalline powder. It shows polymorphism (2.5.11).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lorazepam IPRS* or with the reference spectrum of lorazepam.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *methanol* and 25 volumes of *water.*

Test solution. Dissolve 32 mg of substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). A 0.0032 per cent w/v solution of *lorazepam IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.32 per cent w/v of *lorazepam IPRS* and 0.0032 per cent w/v, each of, *lorazepam related compound A IPRS, lorazepam related compound B IPRS, lorazepam related compound C IPRS, lorazepam related compound D IPRS* and *lorazepam related compound E IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as YMC-Pack ODS-A),
- column temperature: 5°,
- sample temperature: 4°,
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and 1.2 volumes of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 100 µl.

Name	Relative retention time	Correction factor
Lorazepam	1.0	---
Lorazepam related compound D[1]	1.4	---
Lorazepam related compound A[2]	1.7	---
Lorazepam related compound E[3]	1.9	0.77
Lorazepam related compound C[4]	2.1	---
Lorazepam related compound B[5]	5.5	---

[1]6-chloro-4-(*O*-chlorophenyl)-2-quinazolinecarboxylic acid,

[2]7-chloro-5-(*O*-chlorophenyl)-1,3-dihydro-3-acetoxy-2*H*-1,4-benzodiazepin-2-one,

[3]6-chloro-4-(*O*-chlorophenyl)-2-quinazoline methanol,

[4]6-chloro-4-(*O*-chlorophenyl)-2-quinazolinecarboxaldehyde,

[5]2-amino-2', 5-dichlorobenzophenone.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to lorazepam related compound A and lorazepam related compound E is not less than 1.2 in the chromatogram obtained with reference solution (b), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. Run the chromatogram 7 times the retention time of the principal peak for the test solution. The area of any peak corresponding to lorazepam related compound D and lorazepam related compound E, each of, is not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any peak corresponding to lorazepam related compound A is not more than 0.1 times the area of the principal peak in the chromatogram obtained

with reference solution (a) (0.1 per cent), the area of any peak corresponding to lorazepam related compound C is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any peak corresponding to lorazepam related compound B is not more than 0.01 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent).

Sulphated ash (2.3.18). Not more than 0.3 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g under vacuum at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Test solution. Dissolve 100 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *lorazepam IPRS* in the solvent mixture.

- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Lorazepam Injection

Lorazepam Injection is a sterile solution of Lorazepam in a suitable solvent.

Lorazepam Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lorazepam, $C_{15}H_{10}Cl_2N_2O_2$.

Usual strengths. 2 mg per ml; 4 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Lorazepam related compound B. Not more than 0.1 per cent.

Determine by liquid chromatography (2.4.14).

Buffer solution. 0.05 M ammonium dihydrogen phosphate, adjusted to pH 6.5 with *ammonium hydroxide*.

Solvent mixture. Equal volumes of *methanol* and buffer solution.

Test solution. Dilute a volume of the injection containing 16 mg of Lorazepam to 100.0 ml with the solvent mixture.

Reference solution. A 0.0016 per cent w/v solution of *lorazepam related compound B IPRS* in the solvent mixture. Dilute 1.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Luna C18),
- mobile phase: a mixture of 55 volumes of *methanol* and 45 volumes of buffer solution,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent and signal-to-noise ratio is not less than 10.

Inject the reference solution and the test solution.

Calculate the content of lorazepam related compound B.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing 16 mg of Lorazepam to 100.0 ml with the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *lorazepam IPRS* in *methanol*. Dilute a suitable volume with the mobile phase to obtain a solution containing 0.016 per cent w/v of Lorazepam.

Reference solution (b). A solution containing 0.00032 per cent w/v, each of, *lorazepam related compound C IPRS* and *lorazepam related compound D IPRS* in the mobile phase.

Reference solution (c). A solution containing 0.004 per cent w/v of *lorazepam IPRS* and 0.0032 per cent w/v, each of, *lorazepam related compound C IPRS* and *lorazepam related compound D IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 10-15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS),

- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of *0.05M ammonium dihydrogen phosphate*, adjusted to pH 6.5 with *ammonium hydroxide*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Name	Relative retention time
Lorazepam related compound D[1]	0.7
Lorazepam	1.0
Lorazepam related compound C[2]	2.7

[1]6-chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxylic acid,

[2]6-chloro-4-(*o*-chlorophenyl)-2-quinazolinecarboxaldehyde.

Inject reference solution (a) and (c). The test is not valid unless the resolution between the peaks due to lorazepam related compound D and lorazepam is not less than 1.2 and resolution between the peaks due to lorazepam and lorazepam related compound C is not less than 1.2 in the chromatogram obtained with reference solution (c) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (b) and the test solution. The sum of all the secondary peaks is not more than 4.0 per cent.

NOTE — Do not include as an impurity any peak from the test solution that has a retention time less than that of the lorazepam related compound D peak from reference solution (b).

Bacterial endotoxins (2.2.3). Not more than 100.0 Endotoxin Units per mg of lorazepam.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ in the injection.

Storage. Store in single dose or multiple dose containers, preferably of Type-1 glass, protected from light and in a refrigerator (2° to 8°).

Lorazepam Tablets

Lorazepam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lorazepam, $C_{15}H_{10}Cl_2N_2O_2$.

Usual strengths. 1 mg; 2 mg.

Identification

A. Disperse a quantity of the powdered tablets containing 15 mg of Lorazepam with 40 ml of *acetone*, with the aid of ultrasound for 5 minutes and filter. Evaporate the filtrate to dryness on a water-bath in a current of air. Dissolve the residue in 1ml of *acetone* and add 20 ml of 2,2,4-trimethylpentane. Heat the solution on a hot plate and evaporate to a volume of about 10 ml. Remove the solution from hot plate and evaporate to dryness in a current of air. Dry the residue under vacuum at 60° for 1 hour. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lorazepam IPRS* treated in the same manner or with the reference spectrum of lorazepam.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 500 ml of *water*,
Speed and time. 100 rpm and 30 minutes, 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *lorazepam IPRS* in minimum amount of *ethanol* and dilute with the dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

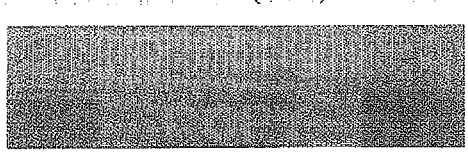
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Luna C18),
- mobile phase: a mixture of 40 volumes of *acetonitrile*, 60 volumes of *water* and 0.4 volume of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ in the medium.

Q. Not less than 60 per cent of the stated amounts of $C_{15}H_{10}Cl_2N_2O_2$ in 30 minutes and not less than 80 per cent of the stated amount of $C_{15}H_{10}Cl_2N_2O_2$ in 60 minutes.

Related substances. Determine by liquid chromatography (2.4.14).



Solvent mixture. 75 volumes of *methanol* and 25 volumes of a buffer solution prepared by dissolving 67.7 g of *sodium acetate trihydrate* in 1000 ml of *water*, adjusted to pH 5.0 with *glacial acetic acid*.

Test solution. Transfer a quantity of powdered tablets containing 20 mg of Lorazepam in 25-ml volumetric flask, add 20 ml of the solvent mixture and stir for 15 minutes. Centrifuge the solution at 2000 rpm for 15 minutes and pass the supernatant through 0.45 µm polyethersulfone membrane filter. Dilute the filtrate with the solvent mixture to obtain a solution containing of 0.016 per cent w/v of lorazepam.

Reference solution (a). A 0.00016 per cent w/v solution of *lorazepam IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.016 per cent w/v of *lorazepam IPRS* and 0.00016 per cent w/v, each of, *lorazepam related compound A IPRS, lorazepam related compound B IPRS, lorazepam related compound C IPRS, lorazepam related compound D IPRS* and *lorazepam related compound E IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as YMC-Pack ODS-A),
- column temperature: 5°,
- sample temperature: 4°,
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 50 volumes of *water* and1.2 volumes of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Lorazepam	1.0	—
Lorazepam related compound D[1]	1.4	—
Lorazepam related compound A[2,*]	1.7	—
Lorazepam related compound E[3]	1.9	0.77
Lorazepam related compound C[4]	2.1	—
Lorazepam related compound B[5]	5.5	—

*Process impurity included for identification and not be included in the total impurities calculation.

[1]6-chloro-4-(*O*-chlorophenyl)-2-quinazolinecarboxylic acid,

[2]7-chloro-5-(*O*-chlorophenyl)-1,3-dihydro-3-acetoxy-2*H*-1,4-benzodiazepin-2-one,

[3]6-chloro-4-(*O*-chlorophenyl)-2-quinazoline methanol,

[4]6-chloro-4-(*O*-chlorophenyl)-2-quinazolinecarboxaldehyde,

[5]2-amino-2',5-dichlorobenzophenone.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to lorazepam related compound A and lorazepam related compound E is not less than 1.2 in the chromatogram obtained with reference solution (b), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to lorazepam related compound D and lorazepam related compound E, each of, is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any peak corresponding to lorazepam related compound C is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent), the area of any peak corresponding to lorazepam related compound B is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Transfer one intact tablet in 10-ml volumetric flask. Add 5 ml of the solvent mixture, disperse with the aid of ultrasound for 10 minutes and shake mechanically for 20 minutes and dilute to volume with the solvent mixture, mix and centrifuge the solution at 2000 rpm for 10 minutes. Dilute the supernatant with the solvent mixture to obtain a solution containing 0.01 per cent w/v of lorazepam.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 85 volumes of *methanol* and 15 volumes of *water*.

Test solution. Weigh 20 intact tablets and transfer to 100-ml volumetric flask. Add 50 ml of the solvent mixture, disperse with the aid of ultrasound for 10 minutes and shake mechanically for 20 minutes and dilute to volume with the solvent mixture, mix and centrifuge the solution at 2000 rpm for 10 minutes. Dilute the supernatant with the solvent mixture to obtain a solution containing 0.01 per cent w/v of lorazepam.

Reference solution. A 0.01 per cent w/v solution of *lorazepam IPRS* in the solvent mixture.

Chromatographic system as described under Dissolution with the following modifications.

– injection volume: 20 µl.

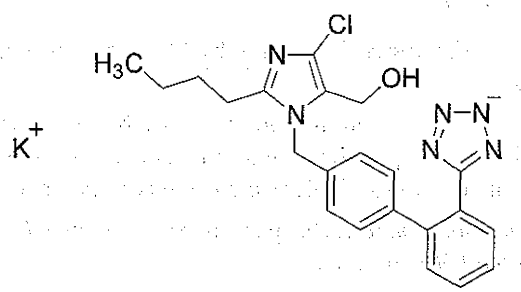
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_{10}Cl_2N_2O_2$ in the tablets.

Storage. Store protected from light and moisture.

Losartan Potassium



$C_{22}H_{22}ClKN_6O$ Mol. Wt. 461.0

Losartan Potassium is monopotassium salt of 2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-methanol.

Losartan Potassium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{22}ClKN_6O$, calculated on the anhydrous basis.

Category. Antihypertensive.

Production

As Nitrosamines are classified as probable human carcinogens, their presence in losartan potassium should be avoided or limited as much as possible. For this reason, manufacturers of losartan potassium for human use are expected to perform an assessment of the risk of *N*-nitrosamine formation and contamination during their manufacturing process; if this assessment identifies a potential risk, the manufacturing process should be modified to minimise contamination and a control strategy implemented to detect and control *N*-nitrosamine impurities in losartan potassium. The general chapter 5.11. Nitrosamine Impurities is available to assist manufacturers.

Description. A white to off-white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *losartan potassium IPRS* or with the reference spectrum of losartan potassium.

B. When examined in the range 200 to 400 nm (2.4.7), a 0.001 per cent w/v solution of *methanol* and compares with the absorbance obtained with a solution of *losartan potassium IPRS* prepared in a similar manner.

C. It gives reaction (A) of potassium (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30 mg of the substance under examination in 100.0 ml of *methanol*.

Reference solution (a). A 0.03 per cent w/v solution of *losartan potassium IPRS* and 0.0002 per cent of *triphenyl-methanol* in *methanol*.

Reference solution (b). A 0.0003 per cent w/v solution of *losartan potassium IPRS* in *methanol*.

Chromatographic system

– a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
– mobile phase: A. 0.1 per cent w/v solution of *orthophosphoric acid* in *water* and filter,
B. *acetonitrile*,
– a gradient programme using the conditions given below,
– flow rate: 1 ml per minute,
– spectrophotometer set at 220 nm,
– injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
35	10	90
50	75	25
55	75	25

The relative retention times are about 1.0 for losartan and 1.9 for triphenylmethanol.

Inject reference solution (a). The test is not valid unless the tailing factor for losartan is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b)

(0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 100.0 ml of *methanol*.

Reference solution. A 0.025 per cent w/v solution of *losartan potassium IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.1 per cent w/v solution of *orthophosphoric acid* in *water* and filter,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
35	10	90
50	75	25
60	75	25

Inject the reference solution. The test is not valid unless the column efficiency in not less than 5000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{22}ClKN_6O$.

Storage. Store protected from moisture.

Losartan Tablets

Losartan Potassium Tablets

Losartan Tablets contain Losartan Potassium.

Losartan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of losartan potassium, $C_{22}H_{22}ClKN_6O$.

Usual strength. 50 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *water*,

Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{22}H_{22}ClKN_6O$ in the medium from the absorbance obtained from a solution of known concentration of *losartan potassium IPRS* in the same medium.

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{22}ClKN_6O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 100 mg of Losartan Potassium in *water* and dilute to 100.0 ml of *water* and filter.

Reference solution (a). A 0.1 per cent w/v solution of *losartan potassium IPRS* in *water*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm packed with octylsilane bonded to porous silica (5 µm), (Such as Lichrosphere RP8e),
- mobile phase: a mixture of 75 volumes of buffer solution prepared by mixing 770 mg of *ammonium acetate* in 1000 ml of *water*, add 2.0 ml of *triethylamine*, adjust pH to 6.5 with *glacial acetic acid* and 25 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 1000 theoretical plates and the tailing factor is not more than 3.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 2 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer intact tablets in a suitable volumetric flask, dissolve in mobile phase and disperse completely. Dilute with mobile phase to obtain a final concentration of 0.0125 per cent w/v.

Reference solution. A 0.125 per cent w/v solution of *losartan potassium IPRS* in mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm packed with octylsilane bonded to porous silica (5 µm), (Such as Lichrosphere RP8e)
- mobile phase: a mixture of 65 volumes of *0.005 M ammonium acetate*, 30 volumes of *acetonitrile*, 5 volumes of *methanol* and 0.2 volumes of *triethylamine*, adjusted to pH 6.6 with *glacial acetic acid* and filter,
- flow rate: 1 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{22}ClKN_6O$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the strength of Losartan Potassium.

Losartan Potassium and Amlodipine Tablets

Amlodipine and Losartan Potassium Tablets; Losartan Potassium and Amlodipine Besylate Tablets; Losartan Potassium and Amlodipine Besilate Tablets

Losartan Potassium and Amlodipine Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of losartan potassium, $C_{22}H_{22}ClKN_6O$ and amlodipine besylate equivalent to amlodipine, $C_{20}H_{25}ClN_2O_5$.

Usual strength. Losartan 50 mg and amlodipine 5 mg.

Identification

In the Assay, the two principle peaks in the chromatogram obtained with the test solution corresponds to the peaks due to losartan potassium and amlodipine in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.01 M sodium acetate solution*, adjusted to pH 4.5 with *glacial acetic acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay, except using injection volume: 50 µl.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). A solution containing 0.25 per cent w/v of *losartan potassium IPRS* and 0.035 per cent w/v of *amlodipine besylate IPRS* in *methanol*.

Reference solution (b). Take a suitable quantity of reference solution (a) to obtain a solution having a known concentration similar to expected concentration of the test solution in the dissolution medium.

Inject reference solution (b) and the test solution.

Calculate the contents of $C_{20}H_{25}ClN_2O_5$ and $C_{22}H_{22}ClKN_6O$ in the medium.

Q. Not less than 70.0 per cent of the stated amounts of $C_{22}H_{22}ClKN_6O$ and $C_{20}H_{25}ClN_2O_5$.

Uniformity of content. *For amlodipine* — Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Assay.

Test solution. Transfer 1 tablet to a 100-ml volumetric flask. Add 5 ml of *water* and sonicate for 10 minutes. Add 70 ml of the mobile phase, sonicate for 10 minutes and dilute to the volume with the mobile phase, filter.

Reference solution. A 0.007 per cent w/v solution of *amlodipine besylate IPRS* in the mobile phase.

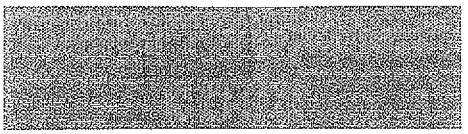
Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 10 intact tablets and transfer to 250-ml volumetric flask. Add 20 ml of *water* and sonicate for 10 minutes. Add about 170 ml of the mobile phase and sonicate for 10 minutes. Make up the volume with the mobile phase, mix and centrifuge. Dilute 5 ml of the supernatant to 25 ml with the mobile phase and filter.



Reference solution. A 0.0055 per cent w/v solution of *amlodipine besylate IPRS* and 0.04 per cent w/v solution of *losartan potassium IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *phosphate buffer pH 5.0*, prepared by dissolving 0.68 g of *potassium dihydrogen orthophosphate* and 4.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 5.0 with *dilute orthophosphoric acid*, 22 volumes of *acetonitrile* and 18 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 237 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless The tailing factor for both the peaks due to amlodipine and losartan potassium is not more than 2.0, the relative standard deviation for replicate injections for each of the peaks corresponding to losartan potassium (first peak) and amlodipine (second peak) is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content $C_{20}H_{25}ClN_2O_5$ and $C_{22}H_{22}ClKN_6O$ in the tablets.

1 mg of Amlodipine besylate is equivalent to 0.000721 g of Amlodipine.

Storage. Store protected from light and moisture.

Labelling. The quantity of Amlodipine besylate is mentioned in the equivalent terms of Amlodipine.

Losartan Potassium and Hydrochlorothiazide Tablets

Hydrochlorothiazide and Losartan Potassium Tablets

Losartan Potassium and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of losartan potassium, $C_{22}H_{22}ClKN_6O$ and hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

Usual strengths. Losartan, 25 mg and hydrochlorothiazide, 5 mg; Losartan, 50 mg and hydrochlorothiazide, 12.5 mg; Losartan, 25 mg and hydrochlorothiazide, 12.5 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the peaks in the chromatogram obtained with reference solution (c) of losartan potassium and hydrochlorothiazide.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). A 0.05 per cent w/v solution of *losartan potassium IPRS* in dissolution medium.

Reference solution (b). Dissolve about 25 mg of *hydrochlorothiazide IPRS* in 10 ml of *acetonitrile* and dilute to 100.0 ml with dissolution medium.

Reference solution (c). Dilute 10.0 ml of reference solution (a) and 5.0 ml of reference solution (b) to 100.0 ml with dissolution medium.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 0.78 g of *sodium dihydrogen orthophosphate* in 500 ml of *water*, adjusted to pH 2.5 with *orthophosphoric acid* and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (c).The test is not valid unless the tailing factor of both the principal peaks is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{22}H_{22}ClKN_6O$ and $C_7H_8ClN_3O_4S_2$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{22}H_{22}ClKN_6O$ and $C_7H_8ClN_3O_4S_2$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic system as described under Dissolution.

For Hydrochlorothiazide —

Test solution. Disperse 1 whole tablet in 100 ml of the mobile phase. Dilute 5 ml of the solution to 50 ml with the mobile phase.

Reference solution. A 0.025 per cent w/v solution of *hydrochlorothiazide IPRS* in the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_7H_8ClN_3O_4S_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 50 mg of Losartan Potassium and 12.5 mg of Hydrochlorothiazide in 100.0 ml of the mobile phase, filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *losartan potassium IPRS* in the mobile phase.

Reference solution (b). A 0.025 per cent w/v solution of *hydrochlorothiazide IPRS* in the mobile phase.

Reference solution (c). Dilute 10.0 ml of reference solution (a) and 5.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Use chromatographic system as described under Dissolution.

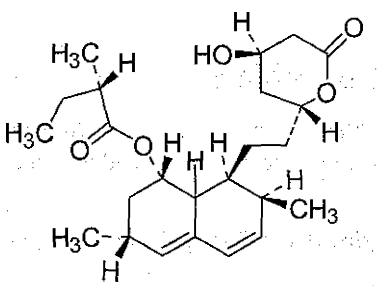
Inject reference solution (c). The test is not valid unless the tailing factor is not more than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{22}H_{22}ClKN_6O$ and $C_7H_8ClN_3O_4S_2$ in the tablets.

Storage. Store protected from light and moisture.

Lovastatin



$C_{24}H_{36}O_5$ Mol. Wt. 404.5

Lovastatin is butanoic acid, 2-methyl-,1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)-ethyl]-1-naphthalenyl ester, [1S-[1α(R*),3α,7β,8β(2S*,4S*),8aβ]]; (S)-2-methylbutyric acid, 8-ester with (4*R*,6*R*)-6-[2-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-1,2,6,7,8,8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthyl] ethyl] tetrahydro-4-hydroxy-2*H*-pyran-2-one.

Lovastatin contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{24}H_{36}O_5$, calculated on the dried basis.

Category. Antihyperlipidemic.

Description. A white to off-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained from *lovastatin IPRS* or with the reference spectrum of lovastatin.

B. When examined in the range 200 to 400 nm (2.4.7), a 0.001 per cent w/v solution in *acetonitrile* shows an absorption maxima at the same wavelength as shown by the reference solution.

Tests

Specific optical rotation (2.4.22). +324° to +338°, determined in a 0.5 per cent w/v solution in *acetonitrile*.

Limit of lovastatin related compound A. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of the *acetonitrile*.

Reference solution(a). A solution containing 0.0002 per cent w/v of *lovastatin IPRS* and *lovastatin related compound A IPRS* in *acetonitrile*.

Reference solution(b). A 0.0002 per cent w/v solution of *lovastatin IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 65 volumes of *acetonitrile* and 35 volumes of *0.01 M ortho phosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Lovastatin related compound A	1.3	0.62
Lovastatin	1.0	—

Inject reference solution (a) and (b). The test is not valid unless the resolution between lovastatin and lovastatin related compound A is not less than 6.0 and the relative standard

deviation for replicate injections is not more than 5.0 per cent with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of lovastatin related compound A is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Chromatographic purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of the *acetonitrile.*

Reference solution(a). A solution containing 0.0002 per cent w/v of *lovastatin IPRS* and *compactin* in *acetonitrile.*

Reference solution(b). A 0.0002 per cent w/v solution of *lovastatin IPRS* in *acetonitrile.*

Chromatographic system

- a stainless steel column 12.5 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- column temperature: 40°,
- mobile phase: A. *0.001 M orthophosphoric acid,* adjusted to pH 4.0 with *1 M sodium hydroxide,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
2	60	40
5	45	55
8	45	55
16	10	90
25	10	90
27	60	40
35	60	40

Name	Relative retention time	Correction factor
Compactin	0.85	—
Lovastatin	1.0	—
Unknown impurity	0.73	0.71

Inject reference solution (a) and (b). The test is not valid unless the resolution between lovastatin and compactin is not less than 3.5, the relative standard deviation for replicate injections is not more than 5.0 per cent with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.04 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 0.3 per cent, determined on 1.0 g by drying in an vacuum oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30 mg of the substance under examination in 100.0 ml of *acetonitrile.*

Reference solution. A 0.03 per cent w/v solution of *lovastatin IPRS* in *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 1 ml of *orthophosphoric acid* in 1000 ml of *water* and 65 volumes of *acetonitrile,*
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{36}O_5$.

Storage. Store protected from moisture, under nitrogen, at a temperature between 2° to 8°.

Lovastatin Tablets

Lovastatin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lovastatin, $C_{24}H_{36}O_5$.

Usual strengths. 10 mg; 20 mg; 40 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Test solution. Disperse a quantity of powder containing 16 mg of lovastatin, add 0.4 ml of *water* and 1.6 ml of *acetonitrile* with the aid of ultrasound. Centrifuge for 4 minutes and use the clear supernatant.

Reference solution. A 0.8 per cent w/v solution of *lovastatin IPRS* in *acetonitrile*.

Mobile phase. A mixture of 5 volumes of *cyclohexane* 2 volumes of *chloroform* and 1 volume of *isopropyl alcohol*.

Apply to the plate 5 µl of each solution . After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of a solution prepared by dissolving 1.38 g of *monobasic sodium phosphate* and 20 g of *sodium lauryl sulphate* in 900 ml of *water*, adjusted to pH 7.0 with *1M sodium hydroxide* and dilute 1000 ml with *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necesssary with the dissolution medium.

Reference solution. Dissolve a quantity of *lovastatin IPRS* in sufficient *methanol* and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 5 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 3.45 g of *monobasic sodium phosphate* in 900 ml of *water* and adjusting the pH 4.0 with *orthophosphoric acid* and dilute with *water* to 1000 ml, 50 volumes of *acetonitrile* and 10 volumes of *methanol*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{36}O_5$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{24}H_{36}O_5$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Test solution. Transfer one tablet in 50-ml volumetric flask, disperse in minimum quantity of *water* and add sufficient quantity of solvent mixture, sonicate and filter. Dilute with solvent mixture to obtain a similar concentration as the reference solution.

Calculate the content of $C_{24}H_{36}O_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.17).

Solvent mixture. 20 volumes of a buffer solution prepared by dissolving 3 ml of *glacial acetic acid* in 900 ml of *water*, adjust to a pH of 4.0 with *sodium hydroxide solution* dilute to 1000 ml with *water* and 80 volumes of *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 40 mg of Lovastatin with 150 ml of solvent mixture with the aid of ultrasound for 20 minutes, cool to room temperature and stand for 30 minutes, dilute to 200.0 ml with the solvent mixture, centrifuge a portion of the solution. Dilute 5.0 ml of supernatant liquid to 25.0 ml with the solvent mixture.

Reference solution. A 0.004 per cent w/v solution of *lovastatin IPRS* in the solvent mixture.

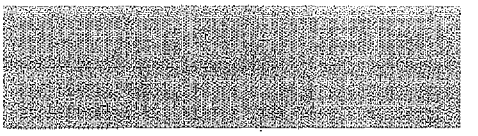
Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 30 volumes of a buffer solution prepared by dissolving 3.45 g of *monobasic sodium phosphate* in 900 ml of *water* and adjusting the pH 4.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*, 50 volumes of *acetonitrile* and 10 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

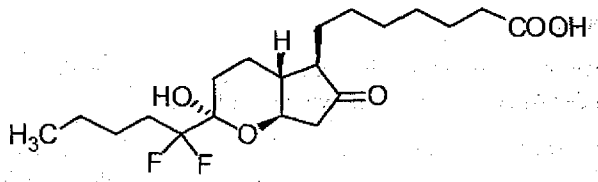
Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{36}O_5$ in the tablets.

Storage. Store protected from light and moisture.



Lubiprostone



$C_{20}H_{32}F_2O_5$ Mol Wt. 390.5

Lubiprostone is 7-[(1*R*,3*R*,6*R*,7*R*)-3-(1,1-Difluoropentyl)-3-hydroxy-8-oxo-2-oxabicyclo[4.3.0]non-7-yl]heptanoic acid.

Lubiprostone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{32}F_2O_5$, calculated on the dried basis.

Category. Constipation agent ,Gastro intestinal drug.

Description. A white to off white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lubiprostone IPRS* or with the reference spectrum of lubiprostone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution. A 0.001 per cent w/v solution of *lubiprostone IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of 0.01 per cent v/v solution of *triflouroaceticacid* in *water* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any scondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of areas of all the secondary peaks is not more than the two times the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak due to lubiprostone tautomer at relative retention time 0.65 with respect to lubiprostone.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 45° for 30 minutes under vacuum at 0.7 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 25.0 ml with the solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *lubiprostone IPRS* in the solvent mixture.

Chromatographic system

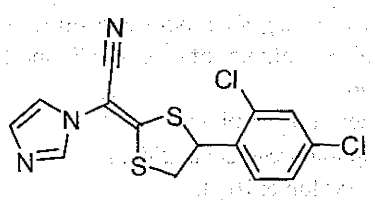
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of 0.01 per cent v/v solution of *triflouroaceticacid* in *water* and 50 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates , the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{32}F_2O_5$.

Luliconazole



$C_{14}H_9Cl_2N_3S_2$ Mol Wt. 354.3

Luliconazole is (2E)[4-(2,4-Dichlorophenyl)-1,3-dithiolan-2-ylidene](1H-imidazol-1-yl)acetonitrile.

Luliconazole contains not less than 98.5 per cent and not more than 102.0 per cent of the stated amount of luliconazole, $C_{14}H_9Cl_2N_3S_2$, calculated on the dried basis.

Category. Antifungal.

Description. An off white to pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *luliconazole IPRS* or with the reference spectrum of luliconazole.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *acetone* is clear (2.4.1) and not more intensely coloured than reference solution BYS5 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

A. *For Luliconazole S-E form* —

Test solution. Dissolve 100 mg of the substance under examination in 70 ml of *acetonitrile* with the aid of ultrasound and dilute to 100.0 ml with the same solvent.

Reference solution. A solution containing 0.002 per cent w/v each of *luliconazole IPRS* and *luliconazole S-E form IPRS ((2E)-[(4S)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene](1H-imidazol-1-yl)ethanenitrile)* in *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with OD-H (5 µm) (Such as Chiralcel OD-H),
- column temperature: 40°,
- mobile phase. a mixture of 20 volumes of 7.0 per cent w/v solution of *sodium perchlorate monohydrate* and 80 volumes of *methanol*,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 10 µl.

The relative retention time with reference to luliconazole for luliconazole S-E form is about 1.18.

Inject the reference solution. The test is not valid unless the resolution between luliconazole peak and luliconazole S-E form peak is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of peak due to luliconazole S-E form is not more than 0.5 times the area of corresponding peak in the chromatogram obtained with the reference solution (1.0 per cent).

B. *For Luliconazole Z form* and other related substances

Test solution. Dissolve 100 mg of the substance under examination in 70 ml of *acetonitrile* with the aid of ultrasound and dilute to 100.0 ml with *acetonitrile.*

Reference solution. A solution containing 0.004 per cent w/v each of *luliconazole IPRS* and *luliconazole Z form IPRS ((2Z)-[4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene](1H-imidazol-1-yl)ethanenitrile)* in *acetonitrile.*

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-2),
- column temperature: 40°,
- mobile phase:a mixture of 54 volumes of a buffer solution prepared by dissolving 2.4 gm of *sodium 1-undecane sulphonate* in 1000 ml of *water,* 45 volumes of *acetonitrile* and 1 volume of *glacial acetic acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 10 µl.

The relative retention time with reference to luliconazole for luliconazole Z form is about 0.86.

Inject the reference solution.The test is not valid unless the resolution between luliconazole peak and luliconazole Z form peak is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with test solution the area of peak due to luliconazole Z form is not more than 0.025 times the area of corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all other secondary peaks other than Z form is not more than 0.025 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Heavy metals (2.3.13). 2.0 g complies with the test for heavy metals, Method B (10 ppm).

Sulphated ash (2.4.18). Not more than 0.1 per cent.

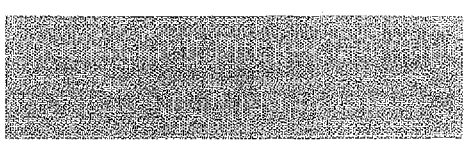
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 105° for 2 hours.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Internal standard solution. A 0.05 per cent w/v solution of *ethyl 4-aminobenzoate* in *acetonitrile.*

Test solution. Dissolve 100 mg of the substance under examination in 70 ml of *acetonitrile* with the aid of ultrasound



and dilute to 100.0 ml with the same solvent. Transfer 10.0 ml of the solution to 50-ml volumetric flask, add 10.0 ml of the internal standard solution and dilute to volume with *acetonitrile*.

Reference solution. A 0.1 per cent w/v solution of *luliconazole IPRS* in *acetonitrile*. Transfer 10.0 ml of the solution to 50-ml volumetric flask, add 10.0 ml of the internal standard solution and dilute to volume with *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with OD-H (5 µm) (Such as Chiralcel OD-H),
- column temperature: 40°,
- mobile phase: a mixture of 60 volumes of *n-hexane*, 40 volumes of *propan-2-ol* and 0.1 volume of *diethylamine*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to the internal standard and luliconazole is not less than 10 and the relative standard deviation of the ratio of peak area of luliconazole to that of peak area of the internal standard, for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_9Cl_2N_3S_2$ using ratio of the peak area of luliconazole to that of peak area of the internal standard.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Luliconazole Cream

Luliconazole Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of luliconazole, $C_{14}H_9Cl_2N_3S_2$.

Usual strength. 1.0 per cent w/w.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.5 to 7.0, determined in 10 per cent w/v solution of cream.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

A. *For Luliconazole S-E form* —

Test solution. Disperse a quantity of cream containing 10 mg of Luliconazole in 70 ml of *acetonitrile*, with the aid of ultrasound, for 10 minutes with intermittent shaking and dilute to 100.0 ml with *acetonitrile*, mix and filter.

Reference solution. A 0.001 per cent w/v solution, each of *luliconazole IPRS and luliconazole S-E form IPRS ((2E)-[(4S)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene](1H-imidazol-1-yl)ethanenitrile))* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with OD-H (5 µm) (Such as Chiralcel OD-H),
- column temperature: 40°,
- mobile phase: a mixture of 20 volumes of 7.0 per cent w/v solution of *sodium perchlorate monohydrate* and 80 volumes of *methanol*,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 10 µl.

The relative retention time with reference to luliconazole for luliconazole S-E form is about 1.17.

Inject the reference solution. The test is not valid unless the resolution between luliconazole peak and luliconazole S-E form peak is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to luliconazole S-E form is not more than 0.25 times the area of the corresponding peak in the chromatogram obtained with the reference solution (2.5 per cent).

B. *For Luliconazole Z form* and other related substances.

Test solution. Disperse a quantity of cream containing about 10 mg of Luliconazole in 70 ml of *acetonitrile*, with the aid of ultrasound for 10 minutes with intermittent shaking and dilute to 100.0 ml with *acetonitrile*, filter.

Reference solution. A 0.001 per cent w/v solution each of *luliconazole IPRS* and *luliconazole Z form IPRS ((2Z)-[4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene](1H-imidazol-1-yl)ethanenitrile))*in *acetonitrile*.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-2),
- column temperature: 40°,
- mobile phase: a mixture of 54 volumes of a buffer solution prepared by dissolving 2.4 gm of *sodium 1-undecanesulphonate* in 1000 ml of *water*, 45 volumes of *acetonitrile* and 1 volume of *glacial acetic acid*,
- flow rate: 1 ml per minute,

- spectrophotometer set at 295 nm,
- injection volume: 10 µl.

The relative retention time with reference to luliconazole for luliconazole Z form is about 0.86.

Inject the reference solution. The test is not valid unless the resolution between luliconazole peak and luliconazole Z form peak is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to luliconazole Z form is not more than 0.03 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.3 per cent) and sum of the areas of all other secondary peaks is not more than 0.03 times the area of the luliconazole peak in the chromatogram obtained with the reference solution (0.3 per cent).

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Internal standard solution. A 0.2 per cent w/v solution of *4-chlorobenzophenone* in *acetonitrile.*

Test solution. Disperse a quantity of cream containing 10 mg of Luliconazole in 50 ml of *acetonitrile,* add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *acetonitrile.*

Reference solution. A 0.1 per cent w/v solution of *luliconazole IPRS* in *acetonitrile.* To 10.0 ml of the solution, add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with OD-H (5 µm) (Such as Chiralcel OD-H 5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 20 volumes of 7.0 per cent w/v solution of *sodium perchlorate monohydrate* and 80 volumes of *methanol,*
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to the internal standard and luliconazole is not less than 9 and the relative standard deviation of the ratio of peak area of luliconazole to that of peak area of the internal standard, for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_9Cl_2N_3S_2$ in the cream using ratio of the peak area of luliconazole to that of peak area of the internal standard.

Storage. Store at a temperature not exceeding 30°.

Luliconazole Lotion

Luliconazole Lotion contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of luliconazole, $C_{14}H_9Cl_2N_3S_2$.

Usual strength. 1.0 per cent w/v.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

A. *For Luliconazole S-E form —*

Test solution. Disperse a quantity of lotion containing 10 mg of Luliconazole in 70 ml of *acetonitrile,* with the aid of ultrasound for 10 minutes with intermittent shaking and dilute to 100.0 ml with *acetonitrile,* mix and filter.

Reference solution. A 0.001 per cent w/v solution, each of *luliconazole IPRS and luliconazole S-E form IPRS ((2E)-[(4S)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene](1H-imidazol-1-yl)ethanenitrile))* in *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with OD-H (5 µm) (Such as Chiralcel OD-H),
- column temperature: 40°,
- mobile phase: a mixture of 20 volumes of 7.0 per cent w/v solution of *sodium perchlorate monohydrate* and 80 volumes of *methanol,*
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 10 µl.

The relative retention time with reference to luliconazole for luliconazole S-E form is about 1.17.

Inject the reference solution. The test is not valid unless the resolution between luliconazole peak and luliconazole S-E form peak is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to luliconazole S-E form is not more than 0.4 times the area of the corresponding peak in the chromatogram obtained with the reference solution (4.0 per cent).

B. *For Luliconazole Z form* and other related substances

Test solution. Disperse a quantity of lotion containing about 10 mg of Luliconazole in 70 ml of *acetonitrile,* with the aid of ultrasound for 10 minutes with intermittent shaking and dilute to 100.0 ml with *acetonitrile,* mix and filter.

Reference solution. A 0.001 per cent w/v solution each of *luliconazole IPRS* and *luliconazole Z form IPRS ((2Z)-[4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene](1H-imidazol-1-yl)ethanenitrile))* in *acetonitrile.*

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-2),
- column temperature: 40°,
- mobile phase: a mixture of 54 volumes of a buffer solution prepared by dissolving 2.4 gm of *sodium 1-undecane sulphonate* in 1000 ml of *water*, 45 volumes of *acetonitrile* and 1 volume of *glacial acetic acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 10 µl.

The relative retention time with reference to luliconazole for luliconazole Z form is about 0.86.

Inject the reference solution. The test is not valid unless the resolution between luliconazole peak and luliconazole Z form peak is not less than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to luliconazole Z form is not more than 0.05 times the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent) and sum of the areas of all other secondary peaks is not more than 0.04 times the area of the luliconazole peak in the chromatogram obtained with the reference solution (0.4 per cent).

Other tests. Comply with the tests stated under Lotions.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Internal standard solution. A 0.2 per cent w/v solution of *4- chlorobenzophenone* in *acetonitrile.*

Test solution. Disperse a quantity of lotion containing 10 mg of Luliconazole in 50 ml of *acetonitrile,* add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *acetonitrile.*

Reference solution. A 0.1 per cent w/v solution of *luliconazole IPRS* in *acetonitrile.* To 10.0 ml of the solution, add 10.0 ml of the internal standard solution and dilute to 100.0 ml with *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with OD-H (5 µm) (Such as Chiralcel OD-H),
- column temperature: 40°,
- mobile phase: a mixture of 20 volumes of 7.0 per cent w/v solution of *sodium perchlorate monohydrate* and 80 volumes of *methanol,*
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume: 10 µl.

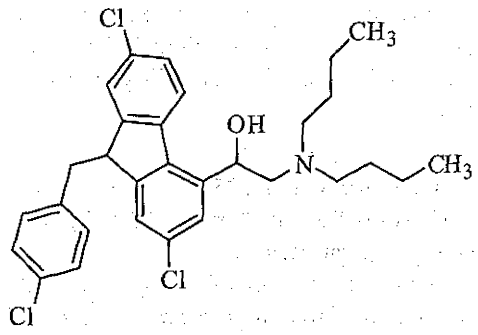
Inject the reference solution. The test is not valid unless the resolution between the peaks due to the internal standard and luliconazole is not less than 9 and the relative standard deviation of the ratio of peak area of luliconazole to that of peak area of the internal standard, replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_9Cl_2N_3S_2$ in the lotion using ratio of the peak area of luliconazole to that of peak area of the internal standard.

Storage. Store at a temperature not exceeding 30°.

Lumefantrine



$C_{30}H_{32}Cl_3NO$ Mol. Wt. 528.9

Lumefantrine is (±)-2,7-Dichloro-9-[(z)-p-chlorobenzylidine]-á[(dibutylamino)methyl]-fluorene-4-methanol.

Lumefantrine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{30}H_{32}Cl_3NO$.

Category. Antimalarial.

Description. A yellow crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lumefantrine IPRS* or with the reference spectrum of lumefantrine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. A 10 per cent w/v solution in *dichloromethane* is not more opalescent than opalescence standard OS1 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modification.

Reference solution (d). Dissolve 5 mg of *lumefantrine IPRS* in 10 ml of *dichloromethane* and dilute to 100.0 ml with *acetonitrile*. Dilute 1.0 ml of the solution to 50.0 ml with *acetonitrile*.

Name	Relative retention time	Correction factor
Desbutyl lumefantrine[1]	0.68	0.91
Lumefantrine	1.0	---

[1](z)-2-(Butylamino)-1-(2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl)ethanol.

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of desbutyl lumefantrine peak is not more than the 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.3 per cent). Ignore any peak other than desbutyl lumefantrine peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavymetals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 10 ml of *dichloromethane* and dilute to 100.0 ml with *acetonitrile*.

Reference solution (a). Dissolve 100 mg of *lumefantrine IPRS* in 10 ml of *dichloromethane* and dilute to 100.0 ml with *acetonitrile*.

Reference solution (b). Dissolve 5 mg of *lumefantrine related compound A IPRS* in 10 ml of *dichloromethane* and dilute to 100.0 ml with *acetonitrile*. Dilute 2.0 ml of the solution to 10.0 ml with *acetonitrile*.

Reference solution (c). Dissolve 10 mg of *lumefantrine IPRS* in 1 ml of *dichloromethane*, add 1.0 ml of reference solution (b) and dilute to 10.0 ml with *acetonitrile*.

Chromatographic system

- a stainless steel column 5 cm × 4.6 mm, packed with octadecylsilanebonded to porous silica (1.8 µm),
- column temperature: 50°,
- mobile phase: A. a mixture of 30 volumes of *acetonitrile* and 70 volumes of a buffer solution prepared by dissolving 5.65 g of *sodium 1-hexanesulphonate* and 2.75 g of *monobasic sodium phosphate* in 900 ml of *water*, adjusted to pH 2.3 with *phosphoric acid* and dilute to 1000 ml with *water*,
  B. a mixture of 54 volumes of *acetonitrile* and 46 volumes of *2-propanol*,
- a gradient programme using the conditions given below,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 2.5 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
1.2	65	35
6.0	50	50
6.4	30	70
10	25	75
15	10	90
15.1	65	35
20	65	35

Name	Relative retention time
Lumefantrine related compound A[1]	0.9
Lumefantrine	1.0

[1](RS, Z)-2-(Dibutylamino)-2-(2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl) ethanol).

Inject reference solution (a) and (c). The test is not valid unless the resolution between the peaks due to lumefantrine and lumefantrine related compound A is not less than 1.3 in the chromatogram obtained with reference solution (c), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent in the chromatogram obtained with reference solution (a).

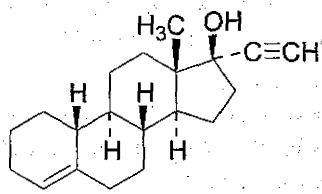
Inject reference solution (a) and the test solution.

Calculate the content of $C_{30}H_{32}Cl_3NO$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Lynoestrenol

Lynestrenol



$C_{20}H_{28}O$ Mol. Wt. 284.4

Lynoestrenol is 19-nor-17α-pregn-4-en-20-yn-17β-ol.

Lynoestrenol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{28}O$, calculated on the dried basis.

Category. Progestogen.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *lynoestrenol IPRS* or with the reference spectrum of lynoestrenol.

B. In the test of Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Melting range. 161° to 165° (2.4.21).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1) and colourless (2.4.1).

Specific optical rotation (2.4.22). – 11.0° to – 9.5°, determined in a 3.6 w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *n-heptane* and 20 volumes of *acetone*.

Test solution (a). Dissolve 0.5 g of the substance under examination in 100.0 ml of *chloroform*.

Test solution (b). Dissolve 0.25 g of the substance under examination in 100.0 ml of *chloroform*.

Reference solution (a). A 0.0025 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution (b). A 0.25 per cent w/v solution of *lynoestrenol IPRS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *0.25 M ethanolic sulphuric acid*, heat at 105° for 10 minutes and examine under ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

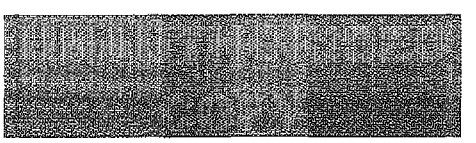
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.5g by drying in an oven at 105°.

Assay. Weigh 0.15 g, dissolve in 40 ml of *tetrahydrofuran*, add 5 ml of a 10 per cent w/v solution of *silver nitrate*. Titrate with *0.1 M sodium hydroxide*, deter-mining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02844 g of $C_{20}H_{28}O$.

Storage. Store protected from light and moisture.

M



Magaldrate

$Al_5Mg_{10}(OH)_{31}(SO_4)_2,xH_2O$ Mol. Wt. 1097.4 (anhydrous)

Magaldrate is a chemical combination of aluminium and magnesium hydroxides and sulphates corresponding approximately to the formula $Al_5Mg_{10}(OH)_{31}(SO_4)_2,xH_2O$.

Magaldrate contains not less than 90.0 per cent and not more than 105.0 per cent of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$, calculated on the dried basis.

Category. Antacid.

Description. A white or almost white, crystalline powder.

Identification

A. Dissolve 0.8 g in 20 ml of *3 M hydrochloric acid*, dilute with *water* to 50 ml, add 3 drops of *methyl red solution* and heat to boiling. Add *dilute ammonia solution* until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50 ml of a hot 2 per cent w/v solution of *ammonium chloride*, then dissolve the precipitate in 15 ml of *3 M hydrochloric acid*; the solution gives the reactions of aluminium salts (2.3.1).

Tests

Arsenic (2.3.10). To 1.0 g add 15 ml of *hydrochloric acid*, 0.1 ml of *stannous chloride solution AsT* and 5 ml of *potassium iodide solution* and allow to stand for 15 minutes. The resulting solution complies with the limit test for arsenic (10 ppm).

Heavy metals (2.3.13). Dissolve 0.33 g in 10 ml of *3 M hydrochloric acid*, filtering to get a clear solution and diluting to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (60 ppm).

Soluble chloride. Boil 1.0 g, weighed, with 50.0 ml of *water* for 5 minutes, cool, add *water* to the original volume, mix and filter. To 25.0 ml of the filtrate add 0.1 ml of *potassium chromate solution* and titrate with *0.1 M silver nitrate* until a persistent pink colour is obtained; Not more than 5.0 ml of *0.1 M silver nitrate* is required (3.5 per cent).

Soluble sulphate. Dilute 2.5 ml of the filtrate obtained in the test for Soluble chloride to 40 ml with *water* in a Nessler cylinder, add 1 ml of *3 M hydrochloric acid* and 3 ml of *barium chloride solution*, dilute to 50.0 ml, mix and allow to stand for 10 minutes; any turbidity produced is not greater than that produced by treating 1.0 ml of *0.01 M sulphuric acid* in the same manner (1.9 per cent).

Sodium. Transfer 2.0 g, weighed, to a 100-ml volumetric flask, place in an ice-bath, add 5 ml of *nitric acid* and swirl to dissolve. Allow to warm to room temperature, dilute with *water* to volume and mix. Filter, if necessary, to obtain a clear solution. Dilute 10.0 ml of the filtrate with *water* to 100.0 ml. The emission intensity of the solution, determined by flame photometry (2.4.4), at about 589 nm and corrected for background transmission at about 580 nm, is not greater than that produced by treating similarly a standard solution containing 2.2 µg of Sodium per ml.

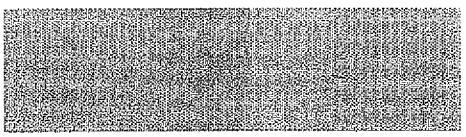
Aluminium hydroxide. 32.1 to 45.9 per cent of $Al(OH)_3$, calculated on the dried basis and determined by the following method. Dissolve about 100 mg, weighed, in 3 ml of *dilute hydrochloric acid* and dilute to 30.0 ml with *water*. Add with stirring 25.0 ml of *0.05 M disodium edetate*, mix and allow to stand for 5 minutes. Add 20 ml of *acetic acid-ammonium acetate buffer*, 60 ml *ethanol (95 per cent)* and 2 ml of *dithizone solution* and titrate with *0.05 M zinc sulphate* to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

Magnesium hydroxide. 49.2 to 66.6 per cent of $Mg(OH)_2$, calculated on the dried basis and determined by the following method. Dissolve about 100 mg, weighed, in 3 ml of *dilute hydrochloric acid* and dilute with *water* to about 200 ml. Add with stirring 1 g of *ammonium chloride*, 20 ml of *triethanolamine*, 10 ml of *ammonia-ammonium chloride buffer* and 0.1 ml of *eriochrome black T solution* and titrate with *0.05 M disodium edetate* to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

Sulphates. 16.0 to 21.0 per cent, calculated on the dried basis and determined by the following method. Weigh 0.875 g, transfer to a 25-ml volumetric flask, dissolve in 10 ml of *water* and 5 ml of *glacial acetic acid*, dilute to volume with *water* and mix. Transfer 5.0 ml of the solution to a glass chromatographic column, 1 cm in internal diameter, prepared by filling with 15 ml of a strongly acidic styrene-divinylbenzene cation exchange resin (50 to 100 mesh) (such as Dowex 50W-X8 or Amberlite 120) and washing the resin with 30 ml of *water*. Elute the column with 15 ml of *water* and collect the eluate in a 125-ml conical flask. To the eluate add 5 ml of a 5.38 per cent w/v solution of *magnesium acetate* and 32 ml of *methanol*. Titrate with *0.05 M barium chloride* using a 0.2 per cent w/v solution of *sodium alizarin sulphonate* as the indicator and adding about 5 ml of the titrant in the beginning and continuing the titration slowly thereafter until the yellow colour disappears and a pink tinge is visible.



1 ml of *0.05 M barium chloride* is equivalent to 0.004803 g of Sulphates.

Microbial contamination (2.2.9). 1.0 g is free from *Escherichia coli*.

Loss on drying (2.4.19). 10.0 to 20.0 per cent, determined on 0.5 g by drying in an oven at 200° for 4 hours.

Assay. Weigh 3.0 g and transfer to a 250-ml conical flask. Add 100.0 ml of *1 M hydrochloric acid* and stir well until a clear solution is obtained. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of *1 M hydrochloric acid* is equivalent to 0.0354 g of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Storage. Store protected from moisture.

Magaldrate Oral Suspension

Magaldrate Suspension

Magaldrate Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous magaldrate, $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Usual strength. The equivalent of 800 mg of anhydrous magaldrate per 5 ml.

Identification

A. Dissolve an amount of the suspension containing about 0.8 g of anhydrous magaldrate in 20 ml of *3 M hydrochloric acid*, dilute with *water* to 50 ml, add 3 drops of *methyl red solution* and heat to boiling. Add *dilute ammonia solution* until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50 ml of a hot 2 per cent w/v solution of *ammonium chloride*, then dissolve the precipitate in 15 ml of *3 M hydrochloric acid*; the solution gives the reactions of aluminium salts (2.3.1).

Tests

Aluminium hydroxide. 32.1 to 45.9 per cent of the stated content of anhydrous magaldrate, determined by the following method. To a quantity containing about 1.0 g of magaldrate add 30 ml of *dilute hydrochloric acid*, shake to dissolve, dilute to 100.0 ml with *water* and mix (solution A). To 10.0 ml of the solution add 20 ml of *water* and Add with stirring 25.0 ml of *0.05 M disodium edetate*, mix and allow to stand for 5 minutes. Add 20 ml of *acetic acid-ammonium acetate buffer*, 60 ml *ethanol (95 per cent)* and 2 ml of *dithizone solution* and titrate with *0.05 M zinc sulphate* to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

Magnesium hydroxide. 49.2 to 66.6 per cent of the stated content of anhydrous magaldrate, determined by the following method. Take 10.0 ml of solution A and dilute with *water* to about 200 ml. Add with stirring 1 g of *ammonium chloride*, 20 ml of *triethanolamine*, 10 ml of *ammonia-ammonium chloride buffer* and 0.1 ml of *eriochrome black T solution* and titrate with *0.05 M disodium edetate* to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

Neutralising capacity. To a weighed quantity of the well-shaken suspension containing 0.8 g of anhydrous magaldrate in a 250-ml beaker add *water* to make a total volume of about 70 ml, heat to 37° and stir continuously, maintaining the temperature at 37°. Add 30.0 ml of *1 M hydrochloric acid* previously heated to 37° and maintain at 37° for 15 minutes, stirring continuously. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.5. Not more than 12 ml of *1 M sodium hydroxide* is required.

Microbial contamination (2.2.9). Total microbial count is not more than 10^2 CFU per ml. 1 ml is free from *Escherichia coli*.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. To a measured volume containing about 3.0 g of anhydrous magaldrate in a beaker add 100.0 ml of *1 M hydrochloric acid* and stir well until a solution is obtained. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of *1 M hydrochloric acid* is equivalent to 0.0354 g of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of mg of anhydrous magaldrate per 5 ml.

Magaldrate Tablets

Magaldrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous magaldrate, $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Usual strength. The equivalent of 800 mg of anhydrous magaldrate.

Identification

To a quantity of the powdered tablets containing about 2 g of anhydrous magaldrate, add about 60 ml of *water*, shake for 3 minutes, centrifuge and discard the supernatant solution. Repeat the washing with three more quantities, each of 60 ml, of *water*. Transfer the residue to a beaker and heat on a water-bath to dryness.

A. Dissolve 0.8 g of the residue in 20 ml of *3 M hydrochloric acid*, dilute with *water* to 50 ml, add 3 drops of *methyl red solution* and heat to boiling. Add *dilute ammonia solution* until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50 ml of a hot 2 per cent w/v solution of *ammonium chloride*, then dissolve the precipitate in 15 ml of *3 M hydrochloric acid*; the solution gives the reactions of aluminium salts (2.3.1).

Tests

Aluminium hydroxide. 32.1 to 45.9 per cent of the stated content of anhydrous magaldrate, determined by the following method. Weigh and finely powder 20 tablets. To a weighed quantity of the powder containing about 1.0 g of anhydrous magaldrate add 30 ml of *dilute hydrochloric acid*, shake well to dissolve, dilute to 100.0 ml with *water* and mix (solution A). To 10.0 ml of the solution add 20 ml of *water* and add with stirring 25.0 ml of *0.05 M disodium edetate*, mix and allow to stand for 5 minutes. Add 20 ml of *acetic acid-ammonium acetate buffer*, 60 ml *ethanol (95 per cent)* and 2 ml of *dithizone solution* and titrate with *0.05 M zinc sulphate* to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

Magnesium hydroxide. 49.2 to 66.6 per cent of the stated content of anhydrous magaldrate, determined by the following method. Take 10.0 ml of solution A and dilute with *water* to about 200 ml. Add with stirring 1 g of *ammonium chloride*, 20 ml of *triethanolamine*, 10 ml of *ammonia-ammonium chloride buffer* and 0.1 ml of *eriochrome black T solution* and titrate with *0.05 M disodium edetate* to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

Neutralising capacity. To a weighed quantity of the powdered tablets containing 0.8 g of anhydrous magaldrate in a 250-ml beaker add about 75 ml of *water* heat to 37° and stir continuously, maintaining the temperature at 37°. Add 30.0 ml of *1 M hydrochloric acid* previously heated to 37° and maintain at 37° for 15 minutes, stirring continuously. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.5 determined potentiometrically (2.4.25). Not more than 12 ml of *1 M sodium hydroxide* is required.

Disintegration (2.5.1). 2 minutes for tablets labelled to be swallowed.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and finely powder 20 tablets. Disperse a quantity of the powder containing about 3.0 g of anhydrous magaldrate in a 200-ml volumetric flask add 100.0 ml of *1 M hydrochloric acid*, shake well for 30 minutes. Dilute to volume and filter. Transfer 100.0 ml to a conical flask. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of *1 M hydrochloric acid* is equivalent to 0.03540 g of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Storage. Store protected from moisture.

Labelling. The label states (1) the strength in terms of the equivalent amount of anhydrous magaldrate; (2) whether the tablets are to be swallowed or chewed.

Magaldrate and Simethicone Chewable Tablets

Magaldrate and Simethicone Chewable Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous magaldrate [$Al_5Mg_{10}(OH)_{31}(SO_4)_2$] and polydimethylsiloxane[$-(CH3)_2SiO-]_n$ not less than 85.0 per cent and not more than 115.0 per cent of the stated amount of simethicone.

Usual strength. Magaldrate 400 mg and Simethicone 60 mg.

Identification

Transfer a quantity of powdered tablets containing 2 g of magaldrate in to a 100 ml centrifuge tube. Add about 60 ml of

water, cap, and shake for 3 minutes. Centrifuge the suspension, and discard the supernatant liquid. Repeat the washing with three more 60-ml portions of *water*. Transfer the residue to a 250-ml beaker, and heat on a water-bath to dryness. The residue complies with the following tests.

A. Dissolve about 0.6 g of magaldrate in 20 ml of *3 M hydrochloric acid*, dilute with *water* to 50.0 ml, add 3 drops of *methyl red solution* and heat to boiling. Add *dilute ammonia solution* until the colour changes to yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 50.0 ml of hot 0.002 per cent w/v solution of *ammonium chloride*, then dissolve the precipitate in 15 ml of *3 M hydrochloric acid*; the solution gives the reactions of aluminium salts (2.3.1).

C. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *polydimethylsiloxane IPRS* or with the reference spectrum of polydimethylsiloxane by using the test solution as described under Assay.

Tests

Neutralising capacity. To a weighed quantity of the powdered tablets containing 0.8 g of anhydrous magaldrate in a 250-ml beaker add about 75 ml of *water* heat to 37° and stir continuously, maintaining the temperature at 37°. Add 30.0 ml of *1 M hydrochloric acid* previously heated to 37° and maintain at 37° for 15 minutes, stirring continuously. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.5 determined potentiometrically (2.4.25). Not more than 12 ml of *1 M sodium hydroxide* is required.

Magnesium hydroxide. 49.2 to 66.6 per cent of $Mg(OH)_2$, of the stated content of anhydrous magaldrate, determined by the following method.

Weigh and powder 20 tablets. Disperse accurately weighed about 1.0 g of powder in 30 ml of *0.01 M hydrochloric acid* and dilute to 100.0 ml with *water* (solution A). Transfer 10.0 ml of solution A to 400-ml beaker, and dilute to about 200 with *water*. Add with stirring 1 g of *ammonium chloride*, 20 ml of *triethanolamine*, 10 ml of *ammonia-ammonium chloride buffer* and 0.1 ml of *eriochrome black T solution* and titrate with *0.05 M disodium edetate* to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

Aluminium hydroxide. 32.1 to 45.9 per cent of $Al(OH)_3$, of the stated content of anhydrous magaldrate, determined by the following method.

To 10.0 ml of the solution prepared for the test of magnesium hydroxide, this solution add 20 ml of *water* and add with stirring 25.0 ml of *0.05 M disodium edetate*, mix and allow to stand for 5 minutes. Add 20 ml of *acetic acid-ammonium acetate buffer*, 60 ml *ethanol (95 per cent)* and 2 ml of *dithizone solution* and titrate with *0.05 M zinc sulphate* to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

Other tests. Comply with the tests stated under Tablets.

Assay.

Magaldrate — Weigh and powder 20 tablets. Disperse a quantity of the powder containing 6.0 g of Magaldrate with 100 ml of *2 M hydrochloric acid*, sonicate for 30 minutes and dilute 200.0 ml with *water*, filter it. Transfer 100.0 ml of the filtrate to a beaker. Titrate with *1 M sodium hydroxide* to pH of 3.0, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *1 M hydrochloric acid* is equivalent to 0.0354 g of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Polydimethylsiloxane —

Test Solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Simethicone, to a 60-ml separator. Add 10.0 ml of *hexanes* and 25 ml of *6 M hydrochloric acid*, cap the separator, and shake by mechanical means for not less than 2 hours. Allow to stand for about 10 minutes, and drain off as much of the lower, aqueous layer as possible without removing any of the unseparated interphase. Add 25 ml of *4 M sodium hydroxide* to the separator, cap it, and shake by mechanical means for 1 hour. Transfer the mixture from the separator to a 50-ml centrifuge tube, cap, and centrifuge to obtain clear layer. Transfer not less than 5 ml of the clear upper *hexanes* layer to a test tube containing about 0.5 g of *anhydrous sodium sulphate*. Cap the tube, shake vigorously, and allow to stand to obtain a clear supernatant liquid.

Reference solution (a). A solution containing 0.16 per cent w/v of *polydimethylsiloxane IPRS* prepare same as that of test solution.

Reference solution (b). A solution containing 0.2 per cent w/v of *polydimethylsiloxane IPRS* prepare same as that of test solution.

Reference solution (c). A solution containing 0.24 per cent w/v of *polydimethylsiloxane IPRS* prepare same as that of test solution.

NOTE — Between each measurement, rinse the cell with heptane, empty, and dry it.

Measure the absorbance by using 0.5 mm cell at the wavelength of maximum absorbance at about 1260 cm^{-1} with an IR spectrophotometer, using *hexane* as the blank. Prepare a standard curve by plotting the absorbance for the standard preparation and draw the straight line best fitting the three plotted points. From this standard curve, determine concentration in mg per ml of sodium polydimethylsiloxane.

Calculate the quantity in mg of $[-(CH3)_2SiO-]_n$ in the tablets by multiplying concentration by 10.

Storage. Store in tightly-closed containers.

Labelling. The Chewable Tablets to indicate that they are to be chewed before being swallowed. The strength in terms of equivalent amount of anhydrous magaldrate.

Magaldrate and Simethicone Oral Suspension

Magaldrate and Simethicone Oral Suspension is a mixture of Magaldrate and Simethicone. It may contain suitable aqueous flavoured vehicle.

Magaldrate and Simethicone Oral Suspensions contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous magaldrate $[Al_5Mg_{10}(OH)_{31}(SO_4)_2]$ and polydimethylsiloxane $[-(CH_3)_2SiO-]_n$ is not less than 85.0 per cent and not more than 115.0 per cent of the stated amount of Simethicone.

Usual strength. Magaldrate 400 mg and Simethicone 60 mg per 5 ml.

Identification

A. Dissolve an amount of Oral Suspension, containing 0.8 g of Magaldrate in 20 ml of *3 M hydrochloric acid*, dilute with *water* to about 50.0 ml, add 3 drops of *methyl red solution*, heat to boiling. Add *6 M ammonium hydroxide* until the colour changes to just yellow, continue boiling for 2 minutes and filter; the filtrate gives the reactions of magnesium salts (2.3.1).

B. Wash the precipitate obtained in test A with 0.002 per cent w/v solution of hot *ammonium chloride* and dissolve the precipitate in *hydrochloric acid*. Divide the resulting solution into two equal portions. The dropwise addition of *6 M ammonium hydroxide* to one portion (Solution 1) yields a gelatinous white precipitate, which does not dissolve in an excess of *6 M ammonium hydroxide*. The dropwise addition of *1 M sodium hydroxide* to the second portion (solution 2) yields a gelatinous white precipitate, which dissolves in an excess of *1 M sodium hydroxide*, leaving some turbidity.

C. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *polydimethylsiloxane IPRS* or with the reference spectrum of polydimethylsiloxane by using the test solution as described in Assay.

Tests

Neutralising capacity. To a weighed quantity of the well-shaken suspension containing 0.8 g of anhydrous magaldrate in a 250-ml beaker add *water* to make a total volume of about 70 ml, heat to 37° and stir continuously, maintaining the temperature at 37°. Add 30.0 ml of *1 M hydrochloric acid* previously heated to 37° and maintain at 37° for 15 minutes, stirring continuously. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.5. Not more than 12 ml of *1 M sodium hydroxide* is required.

Magnesium hydroxide. 49.2 to 66.6 per cent of $Mg(OH)_2$, of the stated amount of anhydrous magaldrate determined by the following method.

To a measured volume containing about 1.0 g of the Magaldrate, in 30 ml of *dilute hydrochloric acid* (1 in 10) and dilute with *water* to about 100 ml (solution A). Transfer 10.0 ml of solution A to 400-ml beaker, and dilute to about 200 with *water*. Add with stirring 1 g of *ammonium chloride*, 20 ml of *triethanolamine*, 10 ml of *ammonia-ammonium chloride buffer* and 0.1 ml of *eriochrome black T solution* and titrate with *0.05 M disodium edetate* to a blue colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of *disodium edetate* required.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

Aluminium hydroxide. 32.1 to 45.9 per cent of $Al(OH)_3$ per g of the stated amount of anhydrous magaldrate, determined by the following method.

Take 10.0 ml of solution A and 20 ml of *water* to a 250 ml beaker and add with stirring 25.0 ml of *0.05 M disodium edetate*, mix and allow to stand for 5 minutes. Add 20 ml of *acetic acid-ammonium acetate buffer*, 60 ml *ethanol (95 per cent)* and 2 ml of *dithizone solution* and titrate with *0.05 M zinc sulphate* to a bright rose-pink colour. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

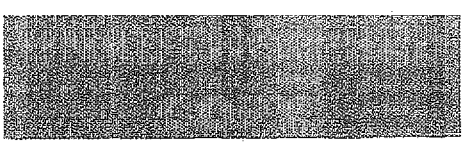
1 ml of *0.05 M disodium edetate* is equivalent to 0.0039 g of $Al(OH)_3$.

Microbial contamination (2.2.9). The total aerobic viable count is not more than 100 CFU per ml. It meets the requirements of the tests for the absence of *Escherichia coli*.

Other tests. Comply with the tests stated under Oral liquids.

Assay

Magaldrate — To a measured volume containing about 3.0 g of Magaldrate in a beaker add 100.0 ml of *1 M hydrochloric*



acid and stir well until a solution is obtained. Titrate the excess acid with *1 M sodium hydroxide* to a pH of 3.0, determined potentiometrically (2.4.25). Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of *1 M hydrochloric acid* is equivalent to 0.0354 g of $Al_5Mg_{10}(OH)_{31}(SO_4)_2$.

Polydimethylsiloxane —

Test Solution. Transfer an accurately measured quantity of Oral Suspension containing about 250 mg of Simethicone, to a 200-ml centrifuge tube. Add an equal volume of *hydrochloric acid*, swirl to dissolve the Oral Suspension, add 25.0 ml of *hexane*, and immediately close the bottle securely with a cap having an inert liner. Shake the bottle for 30 minutes, and centrifuge the mixture until a clear supernatant layer is obtained.

Reference solution (a). A solution containing 1.0 per cent w/v of *polydimethylsiloxane IPRS* in the hexane.

Measure the absorbance by using 0.1 mm cell at the wavelength of maximum absorbance at about 7.9 µm and at the wavelengths of minimum absorbance at about 7.5 µm and 8.3 µm, with a suitable IR spectrophotometer, using hexane as the blank. Draw a linear baseline between the two minima, and determine the absorbance for the reference preparation and the test preparation with respect to the baseline, making any necessary correction for the blank. Calculate the quantity, in mg, of $[-(CH_3)2SiO-]_n$ in the portion of Oral Suspension taken by the formula:

$$25C \times \frac{AU}{AS}$$

in which C is the concentration, in mg per ml of reference and AU and AS are the absorbance of the test preparation and the reference preparation, respectively.

Storage. Store in tightly-closed containers.

Labelling. The strength in terms of equivalent amount of anhydrous magaldrate.

Magnesium Hydroxide

$Mg(OH)_2$ Mol. Wt. 58.3

Magnesium Hydroxide contains not less than 95.0 per cent and not more than 100.5 per cent of $Mg(OH)_2$, calculated on the dried basis.

Category. Antacid; osmotic laxative.

Description. A bulky white powder.

Identification

Dissolve about 15 mg in 2 ml of *2 M nitric acid* and neutralise with *2 M sodium hydroxide*. The resulting solution gives reaction (A) of magnesium salts (2.3.1).

Tests

Appearance of solution. Dissolve 5.0 g in a mixture 50 ml of *5 M acetic acid* and 50 ml of *distilled water*; not more than a slight effervescence is produced. Boil for 2 minutes, cool and dilute to 100.0 ml with *2 M acetic acid*. Filter, if necessary, through a previously ignited and weighed porcelain or silica crucible of a suitable porosity to give a clear filtrate (solution A). Reserve any residue (residue R) for the test for Substances insoluble in *acetic acid*. Solution A is not more intensely coloured than reference solution BS3 (2.4.1).

Arsenic (2.3.10). Dissolve 2.5 g in 18 ml of *brominated hydrochloric acid* and 42 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (4 ppm).

Heavy metals (2.3.13). To 20 ml of solution A add 15 ml of *7 M hydrochloric acid* and shake with 25 ml of *4-methyl-pentan-2-one* for 2 minutes. Separate the layers, evaporate the aqueous layer to dryness, dissolve the residue in 1 ml of *5 M acetic acid* and dilute to 20.0 ml with *water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (40 ppm), using 10 ml of *lead standard solution (2 ppm Pb)*.

Iron (2.3.14). Dissolve 0.2 g in 7 ml of *2 M hydrochloric acid* and dilute to 20.0 ml with *water*. 5 ml of the resulting solution complies with the limit test for iron (0.08 per cent).

Chlorides (2.3.12). 5.0 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for chlorides (0.1 per cent).

Sulphates (2.3.17). 0.6 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for sulphates (0.5 per cent).

Calcium. Not more than 1.5 per cent.

Determine by atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm using nitrous oxide-acetylene flame and calcium hollow-cathode lamp.

Lanthanum solution. Dissolve 58.65 g of lanthanum oxide in 400 ml *water* and add gradually 250 ml *hydrochloricacid*, stir and dilute to 1000.0 ml with *water*.

Blank solution. Dilute 4.0 ml of lanthanum solution and 10.0 ml of *dilute hydrochloric acid* to 200.0 ml with *water*.

Test solution. Dissolve 0.25 g of substance under examination, previously dried, in 30 ml of *dilute Hydrochloric acid*, stir

and heat if necessary. Transfer the solution in a 200-ml volumetric flask containing 4 ml of lanthanum solution, dilute to volume with *water* and mix.

Reference solutions. Dissolve 0.2497 g calcium carbonate, previously dried at 300° for 3 hours and cooled in a desiccator for 2 hours, in a minimum amount of *hydrochloric acid* and dilute to 100.0 ml with *water*. Dilute 1.0 ml, 5.0 ml, 10.0 ml and 15.0 ml of the solution to separate 1000-ml volumetric-flasks, each containing 20.0 ml of lanthanum solution and 40.0 ml of *dilute hydrochloric acid*, dilute with *water* to volume.

Soluble substances. Mix 2.0 g with 100 ml of *water*, boil for 5 minutes, filter whilst hot through a sintered-glass filter (porosity No. 3), allow to cool and dilute to 100.0 ml with *water*. Evaporate 50.0 ml of the filtrate to dryness and dry the residue at 105°; the residue weighs not more than 10 mg (1.0 per cent).

Substances insoluble in acetic acid. Residue R when washed, dried and ignited at 600°, weighs not more than 5.0 mg (0.1 per cent).

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Dissolve 75 mg, previously dried, in 2 ml of 3 *M hydrochloric acid*, and swirl to dissolve. Add 100 ml of *water*, adjust the solution to pH 7 with *1 M sodium hydroxide (using pH indicator paper)*, add 5 ml of *ammonia ammonium chloride buffer* and titrate with *0.05 M disodium edetate* to a blue endpoint, using about 0.15 ml of *eriochrome black T solution* as indicator. Carry out a blank titration.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002916 g of $Mg(OH)_2$.

Storage. Store protected from moisture.

Magnesium Hydroxide Oral Suspension

Magnesium Hydroxide Mixture; Milk of Magnesia; Cream of Magnesia

Magnesium Hydroxide Oral Suspension is an aqueous suspension of hydrated magnesium oxide. It may be prepared from a suitable grade of Light Magnesium Oxide.

Magnesium Hydroxide Oral Suspension contains not less than 7.0 per cent and not more than 8.5 per cent w/w of hydrated magnesium oxide, calculated as $Mg(OH)_2$.

Description. A white, uniform suspension, which does not separate readily on standing.

Identification

A solution of 1 ml of oral suspension in 2 ml of *dilute hydrochloric acid* gives the reactions of magnesium salts (2.3.1).

Tests

Soluble alkalis. Filter about 25 ml and discard the first 10 ml of the filtrate. Dilute 5 ml of the filtrate with 40 ml of *water*, add 0.05 ml of *methyl red solution* and titrate with *0.05 M sulphuric acid* to a persistent pink colour. Not more than 1.0 ml of *0.05 M sulphuric acid* is required.

Soluble salts. To 5 ml of the clear filtrate obtained in the test for Soluble alkalis add 0.15 ml of *sulphuric acid*, evaporate to dryness on a water-bath and then ignite gently to constant weight; the residue does not weigh more than 12 mg.

Heavy metals (2.3.13). Dissolve 12.5 g in 10 ml of *hydrochloric acid* and 20 ml of *water*, add 0.5 ml of *nitric acid*, boil to remove any carbon dioxide and filter. To the cooled filtrate add 2 g of *ammonium chloride* and 2 g of *ammonium thiocyanate* and extract with two successive quantities, each of 10 ml, of *ether*. To the aqueous layer add 2 g of *citric acid* and sufficient *water* to produce 50 ml. 12 ml of the solution complies with the limit test for heavy metals, Method D (4 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Sulphates (2.3.17). Dissolve 2.5 ml in 20 ml of *hydrochloric acid* and dilute to 500.0 ml with *water*. 15 ml of the resulting solution, filtered if necessary, complies with the limit test for sulphates (0.2 per cent).

Microbial contamination (2.2.9). Total microbial count is not more than 10^2 CFU per ml. 1 ml is free from *Escherichia coli*.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Weigh 10.0 g, mix with 50 ml of *water*, add 50.0 ml of *0.5 M sulphuric acid* and titrate the excess of acid with *1 M sodium hydroxide* using *methyl orange solution* as indicator.

1 ml of *0.5 M sulphuric acid* is equivalent to 0.02916 g of hydrated magnesium oxide calculated as $Mg(OH)_2$.

Storage. Store protected from moisture. Do not keep in a refrigerator.

Magnesium Stearate

Magnesium Stearate consists mainly of magnesium stearate $(C_{17}H_{35}CO_2)_2Mg$ with variable proportions of magnesium palmitate, $(C_{15}H_{31}CO_2)_2Mg$ and magnesium oleate, $(C_{17}H_{33}CO_2)_2Mg$.

Magnesium Stearate contains not less than 3.8 per cent and not more than 5.0 per cent of Mg, calculate on the dried basis. The fatty acid fraction contains not less than 40.0 per cent of stearic acid and the sum of stearic acid and palmitic acid is not less than 90.0 per cent.

Category. Pharmaceutical aid (lubricant).

Description. A very fine, light, white powder; unctuous and free from grittiness.

Identification

To 5.0 g add 50 ml of *peroxide-free ether*, 20 ml of *dilute nitric acid* and 20 ml of *water* and heat under a reflux condenser until dissolution is complete. Allow to cool. In a separating funnel, separate the aqueous layer and shake the ether layer with 2 quantities, each of 4 ml, of *water*. Combine the aqueous layers, wash with 15 ml of *peroxide-free ether* and dilute to 50.0 ml with *water* (solution A). Evaporate the organic layer to dryness and dry the residue at 105°.

A. The residue obtained in the preparation of solution A has a freezing point (2.4.11) not less than 53°.

B. The acid value of the fatty acids is 195 to 210, determined on 0.2 g of the residue obtained in the preparation of solution A, dissolved in 25 ml of the prescribed mixture of solvents (2.3.23).

C. In the test for fatty acid composition, the principal peaks in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

D. 1 ml of solution A gives the reaction of magnesium (2.3.1).

Tests

Appearance of solution. Solution A is not more intensely coloured than reference solution YS6 (2.4.1).

Appearance of solution of the fatty acids. Dissolve 0.5 g of the residue obtained in the preparation of solution A in 10 ml of *chloroform*. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS5 (2.4.1).

Acidity or alkalinity. Mix 1.0 g with 20 ml of *carbon dioxide-free water*, boil for 1 minute, shaking continuously, cool and filter. To 10 ml of filtrate add 0.05 ml of *bromothymol blue solution*. Not more than 0.05 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the solution.

Acid value of the fatty acids. 195 to 210, determined on 0.2 g of the residue obtained in the preparation of solution A, dissolved in 25 ml of the prescribed mixture of solvents (2.3.23).

Free stearic acid. Not more than 3 per cent, determined by the following method. Weigh 1.0 g into a stoppered flask, add 50 ml of *chloroform*, stopper the flask and shake well. Filter into a beaker through two thicknesses of filter paper taking care to avoid evaporation of the solvent. Wash the filter with 10 ml of *chloroform* and collect the washings in the beaker. Evaporate the chloroform on a water-bath in a current of air. Dissolve the residue in about 10 ml of *ethanol (95 per cent)* previously neutralised to *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0284 g of stearic acid.

Zinc stearate. Heat 5.0 g with shaking in a mixture of 50 ml of *water* and 50 ml of *dilute sulphuric acid* until the fatty acids separate as an oily layer. Cool, filter the aqueous layer and wash the residue with two successive quantities, each of 5 ml, of hot *water*, combine the filtrate and the washings and dilute to 100.0 ml with *water*. To 5 ml of the resulting solution add 0.5 ml of *ammonium mercurithiocyanate solution* and 0.05 ml of *copper sulphate solution*. Scratch the walls of the container with a glass rod and allow to stand for 15 minutes; no violet precipitate is formed.

Heavy metals (2.3.13). Heat 5.0 g with 40 ml of *2 M acetic acid* and allow to cool. Filter, wash the residue with two quantities, each of 5 ml, of warm *water* and dilute to 100.0 ml with *water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use 10.0 ml of *lead standard solution (1 ppm Pb)* to prepare the standard.

Chlorides (2.3.12). 10.0 ml of solution A diluted to 15 ml complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). Dilute 5.0 ml of solution A to 50.0 ml with *water*. 2.5 ml of the solution diluted to 15 ml with *water* complies with the limit test for sulphates (0.6 per cent)

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.75 g, add 50 ml of a mixture of equal volumes of *1-butanol* and *ethanol*, 5 ml of *strong ammonia solution*, 3 ml of *ammonia buffer pH 10.0*, 30.0 ml of *0.1 M disodium edetate* and 15 mg of *mordant black II mixture*, heat to 45° to 50° and titrate with *0.1 M zinc sulphate* until the colour changes from blue to violet. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of disodium edetate required.

1 ml of *0.1 M disodium edetate* is equivalent to 0.002431 g of Mg.

Fatty acid composition. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 100 mg of the substance under examination in 5 ml of *boron trifluoride solution*. Boil under a reflux condenser for 10 minutes, add 4.0 ml of *heptane* through

the condenser, and add 20.0 ml of a *sodium chloride solution*. Shake and allow the layers to separate. Dry the organic layer over 0.1 g of *anhydrous sodium sulphate*. Dilute 1.0 ml of the solution to 10.0 ml with *heptane*.

Reference solution. Dissolve 50 mg each of *palmitic acid IPRS* and *stearic acid IPRS* in 5.0 ml of *boron trifluoride solution*. Boil under a reflux condenser for 10 minutes, add 4.0 ml of *heptane* through the condenser and add 20.0 ml of a *sodium chloride solution*. Shake and allow the layers to separate. Dry the organic layer over 0.1 g of *anhydrous sodium sulphate*. Dilute 1.0 ml of the solution to 10.0 ml with *heptane*.

Chromatographic system

- a capillary column 30 m x 0.32 mm, packed with fused silica coated with macrogol 20000 (film thickness 0.5 µm);
- temperature:

column	time (min)	temperature (°)
	0-2	70
	2-36	70-240
	36-41	240

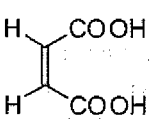
- inlet port at 220° and detector at 260°,
- flame ionization detector,
- flow rate: 2.4 ml per minute, helium or nitrogen as the carrier gas.

Inject 1 µl of the reference solution. The relative retention with reference to methyl stearate for methyl palmitate is about 0.9. The test is not valid unless the resolution between the peaks due to methyl stearate and methyl palmitate is not less than 5.0.

Inject 1 µl of the reference solution and the test solution.

Calculate the percentage content of stearic acid and palmitic acid.

Maleic Acid



$C_4H_4O_4$ Mol. Wt. 116.1

Maleic Acid is (*Z*)-butenedioic acid.

Maleic Acid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_4H_4O_4$, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Description. A white or almost white, crystalline powder.

Identification

A. In the test for fumaric acid, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

B. Dissolve 0.1 g of the substance under examination in 10 ml of *water*. To 0.3 ml of the solution add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*. Heat on a water-bath for 15 minutes, no colour develops. Further to 3 ml of the solution, add 1 ml of *bromine water*. Heat on a water-bath for 15 minutes to remove the bromine, heat to boiling and cool. To 0.2 ml of the solution add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*. Heat on a water-bath for 15 minutes. A violet-pink colour develops.

Tests

Appearance of solution. A 10.0 per cent w/v solution is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

Fumaric acid. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 12 volumes of *anhydrous formic acid*, 16 volumes of *chloroform*, 32 volumes of *butanol* and 44 volumes of *heptane*.

Test solution (a). Dissolve about 0.5 g of the substance under examination in 5.0 ml of *acetone*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 50.0 ml with *acetone*.

Reference solution (a). A 0.2 per cent w/v solution of *maleic acid IPRS* in *acetone*.

Reference solution (b). A 0.15 per cent w/v solution of *fumaric acid IPRS* in *acetone*.

Reference solution (c). A mixture of 5 ml each of reference solution (a) and (b).

Apply to the plate 5 µl of the test solution (a), (b), reference solution (a) and (b) and 10 µl of reference solution (c). Allow the mobile phase to rise 10 cm. Dry the plate at 100° for 15 minutes and examine under ultraviolet light at 254 nm (2.4.7). Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Iron (2.3.14). To 10 ml of a 10 per cent w/v solution in *water*, add 2 ml of *dilute hydrochloric acid* and 0.05 ml of *bromine water*. After 5 minutes, remove the excess of bromine by passing a current of air and add 3 ml of *potassium thiocyanate solution* and shake. Prepare reference solution at the same time and in the same manner, using a mixture of 5 ml of *iron standard solution (1 ppm Fe)*, 1 ml of *dilute hydrochloric*

acid, 6 ml of *water* and 0.05 ml of *bromine water*. Allow both solutions to stand for 5 minutes. Any red colour in the test solution is not more intense than that in the reference solution (5 ppm).

Heavy metals (2.3.13). Weigh in a silica crucible 2 g of the substance under examination, mix with 0.5 g of *magnesium oxide*. Ignite to dull redness until a homogeneous white or greyish-white mass is obtained. After 30 minutes of ignition if mixture remains coloured, allow to cool, mix using a fine glass rod and repeat the ignition. If necessary repeat the operation. Heat at 800° for about 1 hour. Dissolve the residue in 5 ml of a mixture of equal volumes of *hydrochloric acid* and *water*. Add 0.1 ml of *phenolphthalein solution* and then *concentrated ammonia* until a pink colour is obtained. Cool, add *glacial acetic acid* until the solution is decolourised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to 20 ml with *water*, 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (10 ppm), using 10 ml of *lead standard solution (1 ppm pb)* in a mixture of equal volumes of *hydrochloric acid* and *water*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

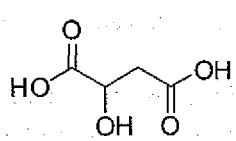
Water (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

Assay. Dissolve 0.5 g in 50 ml of *water*. Titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.05804 g of $C_4H_4O_4$.

Storage. Store protected from light.

Malic Acid



$C_4H_6O_5$ Mol. Wt. 134.1

Malic Acid is (*RS*)-hydroxybutanedioic acid.

Malic Acid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_4H_6O_5$, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *malic acid IPRS* or with the reference spectrum of malic acid.

Tests

Appearance of solution. A 20.0 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

Optical rotation (2.4.22). −0.10° to +0.10°, determined on 20.0 per cent w/v solution in *water*.

Water insoluble substances. Not more than 0.1 per cent.

Dissolve 25.0 g of the substance under examination in 100 ml of *water*, filter the solution through a tared sintered-glass filter, wash the filter with hot *water* and dry at 105° to constant weight. The residue weighs a maximum of 25 mg.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 100 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dissolve 10 mg of *fumaric acid* and 4 mg of *maleic acid* in 50.0 ml of the mobile phase.

Reference solution (b). Dilute 2.5 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). Dissolve 20 mg of the substance under examination in the mobile phase, add 1.0 ml of reference solution (a) and dilute to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with ion-exclusion resin (9 µm),
- column temperature: 37°,
- mobile phase: *0.005 M sulphuric acid*,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to malic acid and malic acid impurity B is not less than 2.5. The relative retention time with reference to malic acid for fumaric acid (malic acid impurity A) is about 1.5 and for maleic acid (malic acid impurity B) is about 0.8.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to fumaric acid is not more than twice the area of the corresponding peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of the peak due to maleic acid is not more than 0.25 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.05 per cent), the area of any other secondary peak is not more than 0.5 times the area of the peak due to maleic acid in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all secondary peaks is not more than 2.5 times the area of the peak due to maleic acid in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an

area less than 0.1 times the area of the peak due to maleic acid in the chromatogram obtained with reference solution (b) (0.02 per cent).

Heavy metals (2.3.13). Not more than 20 ppm, determined by the following method .Weigh 1 g of the substance under examination in a 100-ml long necked combination flask. Clamp the flask at an angle of 45°, add a sufficient volume of a mixture of 8 ml of *sulphuric acid* and 10 ml of *nitric acid* to moisten the substance, warm gently until the reaction starts, allow the reaction to subside and add additional portions of the same acid mixture, heating after each addition. Increase the amount of heat and boil gently until the solution darkens. Cool, add 2 ml of *nitric acid* and heat again until the solution darkens. Continue the heating, followed by the addition of *nitric acid* until no further darkening occur, then heat strongly until dense, white fumes are produced. Cool, add 5 ml of *water*, boil gently until dense, white fumes are produced and continue heating to reduce to 2-3 ml. Cool and add 5 ml of *water* and examine the colour of the solution. If the colour is yellow, cautiously add 1 ml of strong *hydrogen peroxide solution* and again evaporate until dense, white fumes are produced and reduce to a volume of 2 to 3 ml. If the solution is still yellow in colour, repeat the addition of 5 ml of *water* and 1 ml of *strong hydrogen peroxide solution* until the solution is colourless. Cool, and dilute to 25.0 ml with *water* into a 50-ml Nessler cylinder. Adjusted to pH 3.0 with concentrated *ammonia,* dilute with *water* to 40 ml and mix. Add 2 ml of *buffer solution pH 3.5,* mix and add 1.2 ml of *thioacetamide reagent* mix immediately and dilute to 50.0 ml with *water* and. Any colour produced is not more intense than that produced by treating 2 ml of *lead standard solution* (10 ppm).

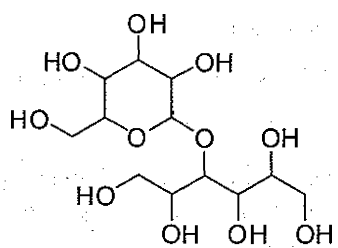
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

Assay. Weigh 0.5 g, dissolved in 50 ml of *water.* Titrate with *1 M sodium hydroxide* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *1 M sodium hydroxide* is equivalent to 0.06705 g of $C_4H_6O_5$.

Maltitol



$C_{12}H_{24}O_{11}$ Mol. Wt. 344.3

Maltitol is α-D-glucopyranosyl-1,4-D-glucitol.

Maltitol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{24}O_{11}$, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Description. A white or almost white crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *maltitol IPRS* or with the reference spectrum of maltitol.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 10 volumes of *water*, 20 volumes of *ethyl acetate* and 70 volumes of *propanol.*

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of *water.*

Reference solution (a). A 0.25 per cent w/v solution of *maltitol IPRS* in *water.*

Reference solution (b). A solution containing 0.25 per cent w/v each of *maltitol IPRS* and *sorbitol IPRS* in *water.*

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in air, spray with *4-aminobenzoic acid solution.* Remove the plate and place it in a current of cold air until the acetone is removed. Heat at 105° for 15 minutes and allow to cool, spray with a 0.2 per cent w/v solution of *sodium periodate.* Dry in a current of cold air. Heat at 100° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

C. Melting point (2.4.21). 148° to 151°.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

Specific optical rotation (2.4.22). + 105.5° to + 108.5°, determined in a 5 per cent w/v solution in *water*, calculated on the anhydrous basis.

Reducing sugars. Not more than 0.2 per cent, expressed as glucose equivalent.

Dissolve 5.0 g in 6 ml of *water* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution* and a few glass beads,

boil for 10 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid* and 94 volumes of *water* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of the titration as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5 g of the substance under examination in 100.0 ml of *water*.

Reference solution (a). A 5.0 per cent w/v solution of *maltitol IPRS* in *water*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

Reference solution (c). Dilute 10.0 ml of reference solution (b) to 100.0 ml with *water*.

Reference solution (d). A solution containing 5.0 per cent w/v each of *maltitol IPRS* and *sorbitol IPRS* in *water*.

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with strong cation exchange resin (calcium form) (9 µm),
- column temperature: 85°,
- mobile phase: *water*,
- flow rate: 0.5 ml per minute,
- refractive index detector,
- injection volume: 20 µl.

The relative retention time with reference to maltitol for sorbitol (maltitol impurity A) is about 1.8, for maltotriitol (maltitol impurity B) is about 0.8.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to maltitol and maltitol impurity A is not less than 2.0.

Inject reference solution (b), (c) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with the area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Lead (2.3.15). Not more than 0.5 ppm.

Nickel. Dissolve 10.0 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and 10 ml of *6 M ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *ethanol* (*95 per cent*). Mix, dilute 50.0 ml with *water* and allow to stand for 5 minutes; any colour produced is not more intense than that produced by treating in the same manner and at the same time 1.0 ml of *nickel standard solution* (*10 ppm Ni*) diluted to 20.0 ml with *water* (1 ppm).

Water (2.3.43). Not more than 1.0 per cent, determined on 1 g.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 10^2 CFU per g and total fungal count is not more than 10^2 CFU per g determined by plate count. 1 g is free from *Escherichia coli* and 10 g is free from *Salmonella* and *Shigella*.

Bacterial endotoxins (2.2.3). Not more than 4 Endotoxin Unit per g for parenteral dosage forms having a concentration of less than 100 g per litre of maltitol and not more than 2.5 Endotoxin Unit per g for parenteral dosage forms having a concentration of less than 100 g per litre or more of maltitol.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{12}H_{24}O_{11}$.

Labelling. The label states (a) where applicable, the maximum concentration of bacterial endotoxins; (b) where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

Liquid Maltitol

Aqueous solution of a hydrogenated, partly hydrolysed starch, composed of a mixture of mainly 4-*O*-α-D-glucopyranosyl-D-glucitol (D-maltitol) with D-glucitol (D-sorbitol) and hydrogenated oligo- and polysaccharides.

Liquid Maltitol contains not less than 50.0 per cent of *D-maltitol*, $C_{12}H_{24}O_{11}$, calculated on the anhydrous basis and 95.0 per cent to 105.0 per cent of the content stated on the label; not more than 8.0 per cent of *D-sorbitol*, $C_6H_{14}O_6$, calculated on the anhydrous basis; not less than 68.0 per cent and not more than 85.0 per cent, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Description. A clear, colourless and syrupy liquid.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal

peak in the chromatogram obtained with reference solution (a).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 10 volumes of *water*, 20 volumes of *ethyl acetate* and 70 volumes of *propanol*.

Test solution. Disperse a volume of solution containing about 0.35 g of maltitol to 100.0 ml with *water*.

Reference solution (a). A 0.2 per cent w/v solution of *maltitol IPRS* in *water*.

Reference solution (b). A solution containing 0.2 per cent w/v each of *maltitol IPRS* and *sorbitol IPRS* in *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in air, spray with *4-aminobenzoic acid solution*. Remove the plate and place it in a current of cold air until the acetone is removed. Heat at 105° for 15 minutes and allow to cool, spray with a 0.2 per cent w/v solution of *sodium periodate*. Dry in a current of cold air. Heat at 100° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

C. To 3 ml of a freshly prepared 10 per cent w/v solution of *pyrocatechol*, add 6 ml of *sulphuric acid* while cooling in iced water. To 3 ml of the cooled mixture, add 0.3 ml of solution A. Heat gently over a naked-flame for about 30 seconds, a pink colour is produced.

Tests

Appearance of solution. A 14.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

Conductivity (2.4.9). Not more than 10 µS cm^{-1}, measured on undiluted liquid maltitol while gently stirring with a magnetic stirrer.

Reducing sugars. Not more than 0.2 per cent, calculated as glucose equivalent.

To 5.0 g add 6 ml of *water*, 20 ml of *cupri-citric solution* and a few glass beads. Heat so that boiling begins after 4 minutes and maintain boiling for 3 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid* and 94 volumes of *water* and when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Lead (2.3.15). Not more than 0.5 ppm.

Nickel. Dissolve 10.0 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and 10 ml of *6 M ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *ethanol* (*95 per cent*). Mix, dilute of 50 ml with *water* and allow to stand for 5 minutes; any colour produced is not more intense than that produced by treating in the same manner and at the same time 1.0 ml of *nickel standard solution* (*10 ppm Ni*) diluted to 20 ml with *water* (1 ppm).

Water (2.3.43). 15.0 per cent to 32.0 per cent, determined on 0.1 g. Use as solvent a mixture of equal volumes of *anhydrous methanol* and *formamide*. Carry out the titration at about 50°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a volume of solution containing about 1.0 g of Maltitol in 50.0 ml of *water*.

Reference solution (a). A 2.0 per cent w/v solution of *maltitol IPRS* in *water*.

Reference solution (b). A 0.32 per cent w/v solution of *sorbitol IPRS* in *water*.

Reference solution (c). An equal mixture of reference solution (a) and reference solution (b).

Chromatographic system
- a stainless steel column 30 cm × 7.8 mm, packed with strong cation exchange resin (calcium form) (9 µm),
- column temperature: 85°,
- mobile phase: *water*,
- flow rate: 0.5 ml per minute,
- refractive index detector,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to sorbitol and maltitol is not less than 2.0. The relative retention time with reference to maltitol for sorbitol is about 1.8.

Inject reference solution (c) and the test solution. Run the chromatograms 3 times the retention time of maltitol.

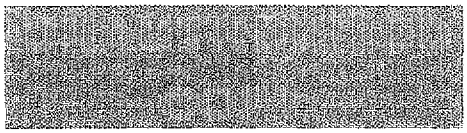
Calculate the content of *D-maltitol*, $C_{12}H_{24}O_{11}$ and *D-sorbitol*, $C_6H_{14}O_6$.

Labelling. The label states the content of D-maltitol.

Maltodextrin

Maltodextrin is a mixture of glucose, disaccharides and polysaccharides, obtained by the partial hydrolysis of starch.

The degree of hydrolysis, expressed as dextrose equivalent (DE), is not more than 20 (nominal value).



Category. Pharmaceutical aid.

Description. A white or almost white, slightly hygroscopic powder or granules.

Identification

A. Dissolve 0.1 g in 2.5 ml of *water* and heat with 2.5 ml of *cupri-tartaric solution*; a red precipitate is produced.

B. Dextrose equivalent (DE) (see Test).

Tests

Solution A. A 25 per cent w/v solution of the substance under examination in *carbon dioxide-free water*.

pH (2.4.24). 4.0 to 7.0, determined in mixture of 1 ml of a 22.36 per cent w/v solution of *potassium chloride* and 30 ml of solution A.

Sulphur dioxide (2.3.40). Not more than 20 ppm.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Dextrose equivalent (DE). Within 2 DE units of the nominal value.

Weigh an amount of the substance under examination equivalent to 2.85 to 3.15 g of reducing carbohydrates, calculated as dextrose equivalent, into a 500-ml volumetric flask. Dissolve in *water* and dilute to 500.0 ml with the same solvent. Transfer the solution to a 50 ml burette. Pipette 25.0 ml of *cupri-tartaric solution* into a 250 ml flask and add 18.5 ml of the test solution from the burette, mix and add a few glass beads. Place the flask on a hot plate, previously adjusted so that the solution begins to boil within 2 minutes ± 15 seconds. Allow to boil for exactly 120 seconds, add 1 ml of a 0.1 per cent w/v solution of *methylene blue* and titrate with the test solution (V_1), until the blue colour disappears. Maintain the solution at boiling throughout the titration. Standardize the cupri-tartaric solution using a 0.6 per cent w/v solution of *glucose* (V_0).

$$\frac{300 \times V_0 \times 100}{V_1 \times M \times D}$$

Where, V_0 = total volume of glucose standard solution, in millilitres,

V_1 = total volume of test solution, in millilitres,

M = sample mass, in grams,

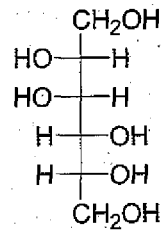
D = percentage content of dry matter in the substance.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 10^3 CFU per g and total fungal count is not more than 10^2 CFU per g determined by plate count. 1 g is free from *Escherichia coli* and 10 g is free from *Salmonella* and *Shigella*.

Labelling. The label states the dextrose equivalent (DE) (nominal value).

Mannitol

D-Mannitol



$C_6H_{14}O_6$ Mol. Wt. 182.2

Mannitol is D-mannitol, a hexahydric alcohol related to mannose.

Mannitol contains not less than 97.0 per cent and not more than 102.0 per cent of $C_6H_{14}O_6$, calculated on the dried basis.

Category. Osmotic diuretic; diagnostic aid (for renal function).

Description. A white, crystalline powder or free-flowing granules.

Identification

A. Determine by infrared absorption spectrophotometery (2.4.6). Compare the spectrum with that obtained with *mannitol IPRS* or with the reference spectrum of mannitol.

B. To 1 ml of a saturated solution add 0.5 ml of *ferric chloride test solution* followed by 0.25 ml of *sodium hydroxide solution* and shake well; a clear solution is obtained which remains clear on the further addition of *sodium hydroxide solution*.

C. Dissolve 5 g in sufficient *carbon dioxide-free water* prepared from *distilled water* to produce 50 ml (solution A). Add 0.3 ml of solution A to 3 ml of a cooled mixture prepared by adding 6 ml of *sulphuric acid* to 3 ml of a freshly prepared 10 per cent w/v solution of *catechol* while cooling in ice. Heat gently over a naked flame for about 30 seconds; a pink colour is produced.

D. Melting range 165° to 170° (2.4.21).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 5 ml of solution A add 5 ml of *carbon dioxide-free water* and 0.05 ml of *dilute phenolphthalein solution*; not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to pink. To a further 5 ml of solution A add 5 ml of *carbon dioxide-free water* and 0.05 ml of *methyl red solution*. Not more than 0.3 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Specific optical rotation (2.4.22). + 23.0° to + 25.0°, determined in a solution prepared by dissolving 2.0 g of the substance under examination and 2.6 g of *sodium tetraborate* in 20 ml of *water* previously heated at 30° and shaking continuously for 15 to 30 minutes without further heating. Dilute the resulting clear solution to 25.0 ml with *water*.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (2 ppm).

Chlorides (2.3.12). A solution of 5.0 g in 10 ml of *water* complies with the limit test for chlorides (50 ppm).

Sulphates (2.3.17). A solution of 1.5 g in 15 ml of *water* complies with the limit test for sulphates (100 ppm).

Reducing sugars. Dissolve 5.0 g in 25 ml of *water* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution* and a few glass beads. Heat so that boiling begins 4 minutes later and continue to boil for 3 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* and 20.0 ml of *0.025 M iodine*. With continuous shaking add 25 ml of a mixture of 6 volumes of *hydrochloric acid* and 94 volumes of *water* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Sorbitol. Determine by thin-layer chromatography (2.4.17), coating the plate with a uniform 0.75-mm layer of the following mixture. Mix 0.1 g of *carbomer* with 110 ml of *water* and allow to stand, with gentle stirring, for 1 hour. Adjusted to pH 7 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A mixture of 85 volumes of *2-propanol* and 15 volumes of a *0.2 per cent w/v solution of boric acid*.

Test solution. Shake 0.5 g of the substance under examination, in fine powder, with 10.0 ml of *ethanol (95 per cent)* for 30 minutes and filter.

Reference solution. A 0.1 per cent w/v solution of *sorbitol IPRS* in *ethanol (95 per cent)*.

Apply to the plate 2 µl of each solution. After development, dry the plate at 100° to 105° for 15 minutes, allow to cool, spray with a 0.5 per cent w/v solution of *potassium permanganate* in *1 M sodium hydroxide* and heat at 100° for 2 minutes. Any spot corresponding to sorbitol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5.0 g of the substance under examination in 25 ml of *water* and dilute to 100.0 ml with *water*.

Reference solution. Dissolve 0.5 g of *mannitol IPRS* in 2.5 ml of *water* and dilute to 10.0 ml with *water*.

Chromatographic system
- a stainless steel column 30 cm x 7.8 mm packed with strong cation-exchange resin (calcium form) (9 µm),
- column temperature: 85 ± 1°,
- mobile phase: degassed *water*,
- flow rate: 0.5 ml per minute,
- refractometer at constant temperature,
- injection volume: 20 µl.

Inject the reference solution and the test solution. Continue the chromatography for twice the retention time of mannitol.

Calculate the content of $C_6H_{14}O_6$.

Mannitol intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 4 Endotoxin Unit per g for parenteral preparation having a concentration of 100 g per litre or less of mannitol, and less than 2.5 Endotoxin Unit per g for parenteral preparations having a concentration of more than 100 g per litre of mannitol.

Labelling. The label states where applicable, the maximum concentration of bacterial endotoxins; whether or not the substance is suitable for use in the manufacture of parenteral preparations.

Storage. Store protected from moisture.

Mannitol Injection

Mannitol Intravenous Infusion

Mannitol Injection is a sterile solution of Mannitol in Water for Injections.

Mannitol Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mannitol, $C_6H_{14}O_6$.

Usual strengths. 10, 15, 20 and 25 per cent w/v.

Description. A colourless or almost colourless clear solution. Particle, if any, present should dissolve on warming.

Identification

A. Evaporate to dryness on a water-bath a volume containing 2.0 g of Mannitol. The residue melts at 165° to 170° (2.4.21).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with the *silica gel G*

Mobile phase. A mixture of 10 volumes of *water*, 70 volumes of *propan-1-ol* and 20 volumes of *ethyl acetate.*

Test solution. Dilute a volume of injection containing 0.25 g of Mannitol to 100 ml with *water.*

Reference solution. A 0.25 per cent w/v solution of *mannitol IPRS* in *water.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with the 0.2 per cent w/v solution of *sodium periodate.* Dry the plate in air for 15 minutes and spray with a 2.0 per cent w/v solution of *4,4'-methylenebis-N,N-dimethylanaline* in a mixture of 1 volume of *glacial acetic acid* and 4 volumes of *acetone,* heat at 105° for 30 minutes and examine under ultraviolet light at 365 nm. The principal spot in chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve 0.5 g of the residue obtained in test A in sufficient *carbon dioxide-free water* prepared from *distilled water* to produce 5 ml (solution A). Add 0.3 ml of solution A to 3 ml of a cooled mixture prepared by adding 6 ml of *sulphuric acid* to 3 ml of a freshly prepared 10 per cent w/v solution of *catechol* while cooling in ice. Heat gently over a naked flame for about 30 seconds; a pink colour is produced.

Tests

pH (2.4.24). 4.5 to 7.0, determined in a solution containing not more than 10.0 per cent w/v solution of Mannitol, diluted if necessary with *water* and to which 0.3 ml of a saturated solution of *potassium chloride* has been added for each 100 ml of solution.

Particulate contamination (2.5.9). When supplied in a container with a nominal content of 100 ml or more, complies with the limit test for particulate contamination.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml of a solution containing not more than 10 per cent w/v of Mannitol. For solutions of higher strength, dilute the injection under examination with *water BET* so that the final solution contains 10 per cent w/v of Mannitol.

Other tests. Comply with the tests stated under Parenteral Preparations (Intravenous Infusions).

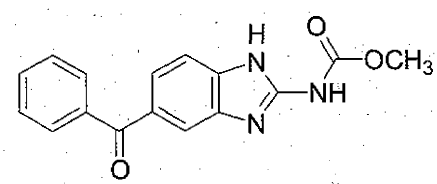
Assay. Dilute a measured volume containing about 0.4 g of Mannitol to 100.0 ml with *water*, transfer 10.0 ml to a stoppered flask, add 20.0 ml of *0.1 M sodium periodate* and 2 ml of *1 M sulphuric acid* and heat on a water-bath for 15 minutes. Cool, add 3 g of *sodium bicarbonate*, in small quantities, and 25.0 ml of *0.1 M sodium arsenite*, mix, add 5 ml of a 20 per cent w/v solution of *potassium iodide* and allow to stand for 15 minutes. Titrate with *0.05 M iodine* until the first trace of yellow colour appears. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of iodine required.

1 ml of *0.05 M iodine* is equivalent to 0.001822 g of $C_6H_{14}O_6$.

Storage. Store at temperatures between 20° and 30°. Exposure to lower temperatures may cause the deposition of crystals, which should be dissolved by warming before use.

Labelling. The label states (1) the strength as a percentage w/v of Mannitol; (2) that the injection should not be used if it contains visible solid particles that do not dissolve on warming.

Mebendazole



$C_{16}H_{13}N_3O_3$ Mol. Wt. 295.3

Mebendazole is Carbamic acid,(5-benzyl-1*H*-binzimidazol-2-yl),methyl ester.

Mebendazole contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{13}N_3O_3$, calculated on the dried basis.

Category. Anthelmintic.

Description. A white to slightly yellow, amorphous powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mebendazole IPRS* or with the reference spectrum of mebendazole.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water.*

Test solution. Dissolve 10 mg of the substance under examination in 160 ml of the solvent mixture, with the aid of ultrasound for 10 minutes at about 50°, cool and dilute to 200.0 ml with the solvent mixture.

Reference solution (a). Dissolve 10 mg of *mebendazole IPRS* in 160 ml of the solvent mixture, with the aid of ultrasound for 10 minutes at about 50°, cool and dilute to 200.0 ml with the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.005 per cent w/v of *mebendazole IPRS* and 0.00025 per cent w/v of *mebendazole related compound D IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- mobile phase: A. a 0.75 per cent w/v solution of *ammonium acetate,*
  B. *acetonitrile,*
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
15	70	30
20	10	90
25	10	90
26	80	20
30	80	20

Name	Relative retention time	Correction factor
2-Amino mebendazole[1]	0.46	—
2-Hydroxy mebendazole[2]	0.53	—
2-Amino-1-methyl mebendazole[3]	0.67	—
Mebendazole	1.0	—
Mebendazole related compound D	1.1	—
Ethyl mebendazole[4]	1.3	—
Toluoyl mebendazole[5]	1.4	—
Mebendazole dimer[6]	1.6	1.41

[1]2-Amino-5-benzoylbenzimidazole,

[2]5-Benzoyl-2-hydroxybenzimidazole,

[3]2-Amino-5-benzoyl-1-methylbenzimidazole,

[4]Ethyl (5-benzoyl-1H-benzimidazol-2-yl)carbamate,

[5]Methyl 5-(4-toluoyl)-1H-benzimidazol-2-ylcarbamate,

[6]1,3-Bis(5-benzoylbenzimidazol-2-yl)urea.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to mebendazole and mebendazole related compound D is not less than 5.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent for mebendazole peak and not more than 5.0 per cent for mebendazole related compound D.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to 2-amino mebendazole, 2-hydroxy mebendazole, 2-amino-1-methyl mebendazole, mebendazole related compound D, ethyl mebendazole and toluoyl mebendazole, each of, is not more than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent), the area of any peak corresponding to mebendazole dimer is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.01 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

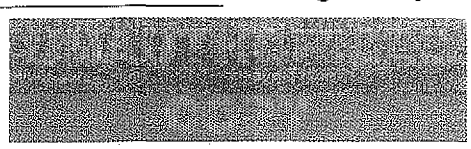
Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a) and (c). The test is not valid unless the resolution between the peaks due to mebendazole and mebendazole related compound D is not less than 5.0 in the chromatogram obtained with reference solution (c), the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{16}H_{13}N_3O_3$.

Storage. Store protected from light and moisture.



Mebendazole Tablets

Mebendazole Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of mebendazole, $C_{16}H_{33}N_3O_3$.

Usual strength. 100 mg.

Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Mebendazole with 10 ml of a mixture of 10 volumes of *anhydrous formic acid* and 90 volumes of *chloroform* for 30 minutes, filter, evaporate the filtrate to dryness and dry the residue at a pressure not exceeding 0.7 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mebendazole IPRS* or with the reference spectrum of mebendazole.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid* containing 1 per cent w/v of *sodium lauryl sulphate,*
Speed and time. 75 rpm and 2 hours.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve 25 mg of *mebendazole IPRS* in 10 ml of *formic acid* and dilute to 50.0 ml with *methanol*. Dilute a volume of the solution with dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 3 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of buffer solution prepared by dissolving 4.0 g of *sodium hydroxide* in 1000 ml of *water,* add 1.5 g of *sodium lauryl sulphate* and 10 ml of *orthophosphoric acid*, adjusted to pH 2.5 with *orthophosphoric acid* and 30 volumes of *acetonitrile,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{13}N_3O_3$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{13}N_3O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Mebendazole in 20 ml of *dimethylformamide,* with the aid of ultrasound for 30 minutes and dilute to 50.0 ml with *dimethylformamide.*

Reference solution (a). A 0.02 per cent w/v solution of *mebendazole IPRS* in *dimethylformamide*. Dilute 1.0 ml of the solution to 100.0 ml with *dimethylformamide.*

Reference solution (b). Dissolve 100 mg of *mebendazole IPRS* and 2 mg of *mebendazole related compound D IPRS* in 100.0 ml of *dimethylformamide*. Dilute 5.0 ml of the solution to 50.0 ml with *dimethylformamide.*

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- mobile phase: A. a 0.75 per cent w/v of *ammonium acetate,*
  B. *acetonitrile,*
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
15	70	30
20	10	90
25	10	90
26	80	20
30	80	20

Name	Relative retention time
Mebendazole	1.0
Mebendazole related compound D[1]	1.1

[1]This is a process-related impurity and not included in the total impurities.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to mebendazole and mebendazole related compound D is not less than 1.5 in the

chromatogram obtained with reference solution (b), and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances using the following solutions.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg of Mebendazole in *dimethylformamide*, with the aid of ultrasound for 30 minutes and dilute to 100.0 ml with *dimethylformamide*. Dilute 5.0 ml of the solution to 50.0 ml with *dimethylformamide*.

Reference solution. A 0.005 per cent w/v solution of *mebendazole IPRS* in *dimethylformamide*.

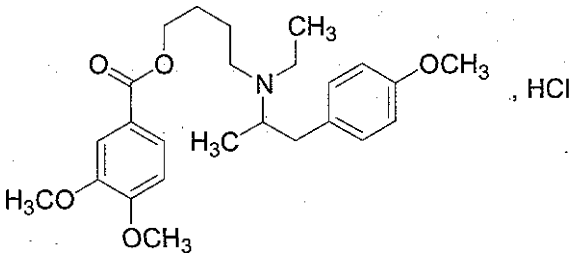
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{13}N_3O_3$ in the tablets.

Storage. Store protected from light and moisture.

Mebeverine Hydrochloride



$C_{25}H_{35}NO_5,HCl$ Mol. Wt. 466.0

Mebeverine Hydrochloride is (*RS*)-4-[ethyl(4-methoxy-α-methylphenethyl)amino]butyl veratrate hydrochloride.

Mebeverine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{25}H_{35}NO_5,HCl$, calculated on the dried basis.

Category. Antispasmodic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mebeverine hydrochloride IPRS* or with the reference spectrum of mebeverine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 263 nm and a less well-defined maximum at about 292 nm; absorbance at about 263 nm, about 0.79 and at about 292 nm, about 0.41.

C. Dissolve 25 mg in 2 ml of *water*, acidify with *2 M nitric acid* and centrifuge. The supernatant liquid gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 2.0 per cent w/v solution.

Ether-soluble extractive. Dissolve 40 mg in 25 ml of *2 M hydrochloric acid* and shake with 50 ml of *ether* for 1 minute. Wash the *ether* layer with three quantities, each of 25 ml, of *water*, evaporate the ether to dryness using a rotary evaporator and dissolve the residue in sufficient *methanol* to produce 20 ml; absorbance of the resulting solution at about 260 nm, not more than 0.23 (2.4.7).

Non-tertiary amine. Dissolve 0.5 g in 5 ml of *pyridine*, add 5 ml of *copper chloride-pyridine reagent* and heat at 50° for 30 minutes. Cool, add sufficient *acetone* to produce 50 ml and measure the absorbance of the resulting solution at about 405 nm (2.4.7), using as the blank a solution obtained by treating 5 ml of *pyridine* in the same manner. The absorbance is not more than that obtained by repeating the test using 5 ml of a 0.006 per cent w/v solution of *di-n-butylamine* in *pyridine* and beginning at the words "add 5 ml of *copper chloride-pyridine reagent....*".

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 50 volumes of *ethanol*, 50 volumes of *chloroform* and 1 volume of *18 M ammonia*.

Test solution. Dissolve 0.2 g of the substance under examination in 10.0 ml of *acetone*.

Reference solution (a). Dissolve 10 mg of the substance under examination in 100.0 ml of *acetone*.

Reference solution (b). A 0.002 per cent w/v solution of *veratric acid* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Expose the plate to iodine vapour for 1 hour. When viewed under ultraviolet light, any spot corresponding to veratric acid in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).Using both methods of visualisation any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

Assay. Dissolve 0.4 g in 75 ml of *anhydrous glacial acetic acid* and add 7 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04660 g of $C_{25}H_{35}NO_5$,HCl.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Mebeverine Tablets

Mebeverine Hydrochloride Tablets

Mebeverine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mebeverine hydrochloride, $C_{25}H_{35}NO_5$,HCl. The tablets are coated.

Usual strength: 135 mg.

Identification

A. Suspend a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride in 20 ml of *water*, add 5 ml of *5 M sodium hydroxide* and extract with two quantities, each of 25 ml, of *chloroform*. Dry the combined extracts over *anhydrous sodium sulphate* and evaporate to dryness using a rotary evaporator.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mebeverine hydrochloride IPRS* treated in the same manner or with the reference spectrum of mebeverine.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum at about 263 nm and a less well-defined maximum at about 292 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 50 volumes of *ethanol*, 50 volumes of *chloroform* and 1 volume of *18 M ammonia*.

Test solution. Disperse a quantity of the powdered tablets containing 0.2 g of Mebeverine Hydrochloride with 10.0 ml of *acetone* and filter.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with *acetone*.

Reference solution (b). A 0.01 per cent w/v solution of *veratric acid* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Expose the plate to iodine vapour for 1 hour. When viewed under ultraviolet light, any spot corresponding to veratric acid in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Using both methods of visualisation any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

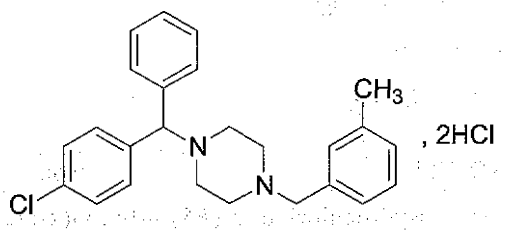
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.5 g of Mebeverine Hydrochloride, dissolve in 100 ml of *0.1 M hydrochloric acid* and heat for 10 minutes on a water-bath, shaking occasionally. Cool, add sufficient *0.1 M hydrochloric acid to* produce 250.0 ml and filter. To 10.0 ml of filtrate add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml and dilute 10.0 ml of the solution to 100.0 ml with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 263 nm (2.4.7). Calculate the content of $C_{25}H_{35}NO_5$,HCl. taking 263 as the specific absorbance at 263 nm.

Storage. Store protected from light and moisture.

Meclizine Hydrochloride

Meclizine Dihydrochloride; Meclozine Hydrochloride; Meclozine Dihydrochloride



$C_{25}H_{27}ClN_2$,2HCl Mol. Wt. 463.9

Meclizine Hydrochloride is (*RS*)-1-(4-chlorobenzhydryl)-4-(3-methylbenzyl)piperazine dihydrochloride.

Meclizine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{25}H_{27}ClN_2,2HCl$, calculated on the anhydrous basis.

Category. Antiemetic.

Description. A white or yellowish white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *meclizine hydrochloride IPRS* or with the reference spectrum of meclizine hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 232 nm and weak absorption without a defined maximum in the range 260 nm to 300 nm; absorbance at the maximum at about 232 nm, 0.51 to 0.57.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Dissolve about 15 mg in 2 ml of *ethanol (95 per cent)*; the solution gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. Equal volumes of *dichloromethane* and *methanol*.

Mobile phase. A mixture of 60 volumes of *dichloromethane*, 30 volumes of *toluene*, 5 volumes of *methanol* and 0.5 volume *of strong ammonia solution*.

Test solution (a). Dissolve 0.5 g of the substance under examination in 10.0 ml of the solvent mixture.

Test solution (b). Dissolve 0.5 g of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). Dissolve 25.0 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (b). A 0.5 per cent w/v solution of *meclizine hydrochloride IPRS* in the solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any yellowish white spot on the line of application.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.2 g.

Assay. Dissolve 0.35 g in 50 ml of *chloroform*, add 50 ml of *anhydrous glacial acetic acid*, 5 ml of *acetic anhydride* and 12 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using a 0.1 per cent w/v solution of *quinaldine red* in *anhydrous glacial acetic acid* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02319 g of $C_{25}H_{27}ClN_2,2HCl$.

Storage. Store protected from light and moisture.

Meclizine Tablets

Meclizine Hydrochloride Tablets; Meclozine Tablets; Meclozine Hydrochloride Tablets; Meclizine Dihydrochloride Tablets; Meclozine Dihydrochloride Tablets.

Meclizine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of meclizine hydrochloride, $C_{25}H_{27}ClN_2,2HCl$.

Usual strength. 25 mg.

Identification

Triturate a quantity of the powdered tablets containing 0.5 g of Meclizine Hydrochloride with three quantities, each of, 15 ml of *chloroform*. Filter the extracts and evaporate the clear filtrate to dryness on a water-bath. The residue complies with test A and C.

A. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *meclizine hydrochloride IPRS* or with the reference spectrum of meclizine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Dissolve 15 mg of the residue in 2 ml of *ethanol (95 per cent)*; the solution gives reaction (A) of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of the mobile phase and dissolution medium.

Test solution. Use the filtrate, dilute if necessary with the mobile phase.

Reference solution. Dissolve a quantity of *meclizine hydrochloride IPRS* in *ethanol* (not more than 1 per cent of volume of flask) and dilute with the solvent mixture to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with irregular or spherical, totally porous silica gel, bonded with strongly acidic cation-exchange coating (3 to 10 µm),
- mobile phase: a mixture of 55 volumes of *water* and 45 volumes of *methanol*, containing 0.69 g of *monobasic sodium phosphate*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{25}H_{27}ClN_2,2HCl$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{25}H_{27}ClN_2,2HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.25 g Meclizine Hydrochloride in the mobile phase, with the aid of mechanical shaker for 30 minutes and dilute to 100.0 ml with the mobile phase, filter.

Reference solution (a). A 0.0025 per cent w/v solution of *meclizine hydrochloride IPRS* in the mobile phase.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with phenyl groups bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 1.32 g of *dibasic ammonium phosphate* in 1000 ml of *water*, adjusted to pH 7.5 with *orthophosphoric acid*, 32.5 volumes of *methanol* and 32.5 volumes of *acetonitrile*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 232 nm,
- injection volume: 20 µl.

The relative retention time with reference to meclizine hydrochloride for 4- chlorobenzophenone is about 0.23.

Inject reference solution (a) and (b). The test is not valid unless the column efficiency is not less than 1200 theoretical plate, the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a) and the signal-to-noise ratio is not less than 10 for the principal peak in the chromatogram obtained with reference solution (b).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 4-chlorobenzophenone, multiplied with correction factor of 1.39, is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a)) (0.1 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 12.5 mg of Meclizine Hydrochloride in the mobile phase, with the aid of mechanical shaker for 30 minutes and dilute to 100.0 ml with the mobile phase, filter.

Reference solution. A 0.0125 per cent w/v solution of *meclizine hydrochloride IPRS* in the mobile phase.

Use the chromatographic system as described under Related substances

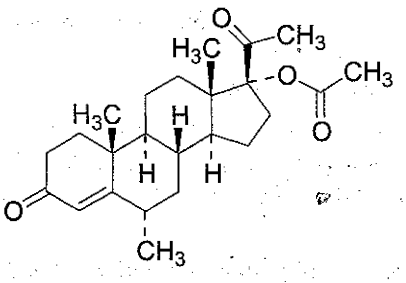
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{27}ClN_2,2HCl$ in the tablets.

Storage. Store protected from light and moisture.

Medroxyprogesterone Acetate



$C_{24}H_{34}O_4$ Mol. wt. 386.5

Medroxyprogesterone Acetate is 6α-methyl-3,20-dioxo-pregn-4-en-17α-yl acetate.

Medroxyprogesterone Acetate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{24}H_{34}O_4$, calculated on the dried basis.

Category. Progestogen.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *medroxyprogesterone acetate IPRS* or with the reference spectrum of medroxyprogesterone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 90 volumes of *chloroform* and 10 volumes of *methanol*

Mobile phase. A mixture of 70 volumes of *toluene*, 40 volumes of *ethyl acetate* and 10 volumes of *light petroleum (50° to 70°)*.

Test solution. Dissolve 0.1 g of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *medroxyprogesterone acetate IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.05 per cent w/v each of *progesterone IPRS* and *medroxyprogesterone acetate IPRS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Spray with *ethanolic sulphuric acid (20 per cent)*, heat at 120° for 10 minutes or until spots appear and allow to cool. Examine the plate under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Melting range 205° to 209° (2.4.21).

Tests

Specific optical rotation (2.4.22). +45.0° to +51.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

Related substances. Determine by liquid chromatography (2.4.17).

Test solution (a). Dissolve 5 mg of the substance under examination in 100.0 ml of the mobile phase.

Test solution (b). Dissolve 0.25 g of the substance under examination in 100.0 ml of the mobile phase.

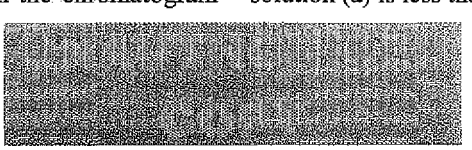
Reference solution. Evaporate 1 ml of a solution containing 0.1 per cent w/v each of *medroxyprogesterone acetate IPRS* and *megestrol acetate IPRS* in *ethanol*, to dryness in a water-bath at 45° and dissolve the residue in sufficient mobile phase to produce 25 ml.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 mm),
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase at a flow rate of 1 ml per minute for about 45 minutes. When the chromatograms are recorded under the conditions described above, the retention times are 12.5 minutes for megestrol acetate and 13.5 minutes for medroxyprogesterone acetate.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to megestrol acetate and medroxyprogesterone acetate is at least 2.0. If this resolution is not achieved, adjust the concentration of *acetonitrile* in the mobile phase. Verify the repeatability by making five separate injections of test solution (a). The system is not suitable unless the relative standard deviation for the area of the principal peak in the chromatogram obtained with test solution (a) is less than 2.0 per cent.



Inject test solution (a) and (b). Record the chromatograms for 1.5 times the retention time of the principal peak. In the chromatogram obtained with test solution (b) the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with test solution (a) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than 0.75 times the area of the principal peak in the chromatogram obtained with test solution (a) (1.5 per cent). Ignore any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with test solution (a) (0.05 per cent).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 25 mg in sufficient *ethanol (95 per cent)* to produce 100.0 ml, dilute 5.0 ml to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7). Calculate the content of $C_{24}H_{34}O_4$ taking 426 as the specific absorbance at 241 nm.

Storage. Store protected from light and moisture.

Medroxyprogesterone Injection

Medroxyprogesterone Acetate Injection

Medroxyprogesterone Injection is a sterile suspension of Medroxyprogesterone Acetate in a suitable vehicle.

Medroxyprogesterone Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of medroxyprogesterone acetate, $C_{24}H_{34}O_4$.

Usual strength. 150 mg per ml.

Identification

Dissolve a volume of the injection containing about 50 mg of Medroxyprogesterone Acetate in 8 ml of *light petroleum (Boiling range 40° to 60°)* and extract with three 8 ml of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *light petroleum (Boiling range 40° to 60°)*, dilute with *water* until the solution becomes turbid. Allow to stand in ice for two hours and filter. Wash the precipitate with *water* and dry at 105°. Determine by infrared absorption spectrophotometry (2.4.6), on the residue. Compare the spectrum with that obtained with *medroxyprogesterone acetate IPRS* or with the reference spectrum of medroxyprogesterone acetate.

Tests

Impurity F (6α-methyl-3,20-dioxo-5β-pregnan-17-yl acetate). Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 10 volumes of *tetrahydrofuran*, 45 volumes of *1,1-dimethylethyl methyl ether* and 45 volumes of *hexane*.

Test solution. Dilute the injection with *dichloromethane*, if necessary, to obtain a 2.0 per cent w/v solution of Medroxyprogesterone Acetate.

Reference solution. A 0.01 per cent w/v solution of *medroxyprogesterone acetate impurity F IPRS* in 2.0 per cent w/v solution of *medroxyprogesterone acetate IPRS* in *dichloromethane*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and carry out a second development in the same direction, using a freshly prepared mobile phase. Dry the plate at 105° and allow to cool, spray with a 20.0 per cent w/v solution of *toluenesulphonic acid* in *ethanol (95 per cent)*. Heat at 120° for 10 minutes. Allow to cool and examine the plate in the ultraviolet light at 365 nm. In the chromatogram obtained with the test solution, any blue fluorescent spot with an R_f value higher than the principal spot is not more intense than the corresponding blue fluorescent spot due to medroxyprogesterone impurity F in the chromatogram obtained with the reference solution (0.5 per cent). The test is not valid unless the chromatogram obtained with the reference solution shows two clearly separated spots.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a quantity of the injection containing about 40 mg Medroxyprogesterone Acetate in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v of *medroxyprogesterone acetate IPRS* and 0.005 per cent w/v of *megestrol acetate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Phenomenex Prodigy ODS3),
- column temperature: 40°,
- mobile phase: a mixture of 10 volumes of *tetrahydrofuran*, 35 volumes of *acetonitrile*, 50 volumes of *water* and diluted to 100 volumes with *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 241 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.3 and the tailing factor of the peak due to medroxyprogesterone acetate is not more than 1.3.

Inject reference solution (a) and the test solution. In the chromatogram obtained with test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of areas of all other secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the injection with mobile phase to obtain 0.004 per cent w/v solution of Medroxyprogesterone Acetate.

Reference solution (a). A 0.004 per cent w/v solution of *medroxyprogesterone acetate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v of *medroxyprogesterone acetate IPRS* and 0.005 per cent w/v of *megestrol acetate IPRS* in the mobile phase.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.3 and the tailing factor of the peak due to medroxyprogesterone acetate is not more than 1.3.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{24}H_{34}O_4$ in the injection.

Storage. Store protected from light.

Medroxyprogesterone Tablets

Medroxyprogesterone Acetate Tablets

Medroxyprogesterone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of medroxyprogesterone acetate, $C_{24}H_{34}O_4$.

Usual strengths. 2.5 mg; 5 mg; 10 mg.

Identification

Disperse a quantity of the powdered tablets containing about 50 mg of Medroxyprogesterone Acetate in 8 ml of *petroleum spirit (boiling range, 40° to 60°)* and extract with three 8 ml quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *petroleum spirit (boiling range, 40° to 60°)*, dilute with *water* until the solution becomes turbid, allow to stand in ice for 2 hours and filter. Wash the precipitate with *water* and dry at 105°. Determine by infrared absorption spectrphotometry (2.4.6), on the residue. Compare the spectrum with that obtained with *medroxyprogesterone acetate IPRS* or with the reference spectrum of medroxyprogesterone acetate.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 0.5 per cent w/v solution of *sodium lauryl sulphate,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium to produce a 0.00028 per cent w/v solution of Medroxyprogesterone Acetate.

Reference solution. A 0.00028 per cent w/v solution of *medroxyprogesterone acetate IPRS* in the dissolution medium.

Chromatographic system
- a stainless steel column 8 cm x 4 mm, packed with octylsilane bonded to porous silica (10 µm) (Such as Zorbax C8),
- mobile phase: a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{34}O_4$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{24}H_{34}O_4$.

Impurity F (6α-methyl-3,20-dioxo-5β-pregnan-17-yl acetate). Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 10 volumes of *tetrahydrofuran*, 45 volumes of *1,1-dimethylethyl methyl ether* and 45 volumes of *hexane.*

Test solution. Shake a quantity of powdered tablets containing about 0.2 g of Medroxyprogesterone Acetate with *dichloromethane* and dilute to 10.0 ml with the same solvent. Centrifuge and use the supernatant liquid.

Reference solution. A 0.01 per cent w/v solution of *medroxyprogesterone acetate impurity F IPRS* in 2.0 per cent w/v solution of *medroxyprogesterone acetate IPRS* in *dichloromethane.*

Apply to the plate 10 µl of each solution. After removal of the plate, dry the plate in air and carry out a second development

in the same direction using a freshly prepared mobile phase. Dry the plate at 105° and allow to cool, spray with a 20 per cent w/v solution of *toluenesulphonic acid* in *ethanol (95 per cent)*. Heat at 120° for 10 minutes, allow to cool and examine the plate under ultraviolet light at 365 nm. In the chromatogram obtained with the test solution, any blue fluorescent spot with an R_f value higher than that of the principal spot is not more intense than the corresponding blue fluorescent spot due to medroxyprogesterone impurity F in the chromatogram obtained with the reference solution (0.5 per cent). The test is not valid unless the chromatogram obtained with the reference solution shows two clearly separated spots.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing about 40 mg of Medroxyprogesterone Acetate in 50 ml of the mobile phase and dilute to 100 ml with the mobile phase and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v of *medroxyprogesterone acetate IPRS* and 0.005 per cent w/v of *megestrol acetate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base-deactivated end-capped octadecylsilane bonded to porous silica (5 µm) (Such as Phenomenex Prodigy ODS3),
- column temperature: 40°,
- mobile phase: a mixture of 10 volumes of *tetrahydrofuran*, 35 volumes of *acetonitrile*, 50 volumes of *water* and dilute to 100 volumes with *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 241 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.3 and the tailing factor of the peak due to medroxyprogesterone acetate is not more than 1.3.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than that 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 40 mg of Medroxyprogesterone Acetate with 50 ml of the mobile phase for 15 minutes, add sufficient mobile phase to produce 100 ml, mix and filter. To 5ml of the filtrate add sufficient mobile phase to produce 50.0 ml.

Reference solution (a). A 0.004 per cent w/v solution of *medroxyprogesterone acetate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v of *medroxyprogesterone acetate IPRS* and 0.005 per cent w/v of *megestrol acetate IPRS* in the mobile phase.

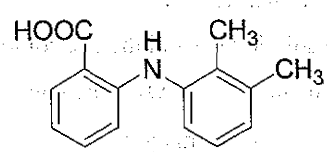
Use chromatographic system as described in the test for Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.3 and the tailing factor of the peak due to medroxyprogesterone acetate is not more than 1.3.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{24}H_{34}O_4$ in the tablets.

Mefenamic Acid



$C_{15}H_{15}NO_2$ Mol. Wt. 241.3

Mefenamic Acid is *N*-(2,3-xylyl)anthranilic acid.

Mefenamic Acid contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{15}H_{15}NO_2$, calculated on the dried basis.

Category. Antiinflammatory; analgesic.

Description. A white to greyish-white, microcrystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mefenamic acid IPRS* or with the reference spectrum of mefenamic acid.

B. Dissolve 25 mg in 15 ml of *chloroform* and examine under ultraviolet light at 365 nm; the solution exhibits a strong greenish-yellow fluorescence. Carefully add 0.5 ml of a saturated solution of *trichloroacetic acid* drop wise and

examine again under ultraviolet light at 365 nm; the solution does not exhibit fluorescence.

C. Dissolve 5 mg in 2 ml of *sulphuric acid* and add 0.05 ml of *0.0167 M potassium dichromate*; an intense blue colour is produced immediately which fades rapidly to brownish-green.

Tests

Light absorption (2.4.7). Absorbance of a 0.002 per cent w/v solution in a mixture of 99 volumes of *methanol* and 1 volume of *1 M hydrochloric acid* at the maximum at about 279 nm, 0.69 to 0.74 and at the maximum at about 350 nm, 0.56 to 0.60.

Copper. Moisten 1.0 g with *sulphuric acid* and ignite until all the carbon is removed. Add 10 ml of *1 M sulphuric acid* to the residue and allow to stand for 10 minutes. Transfer to a separating funnel using 20 ml of *water* and add 10 ml of a solution containing 20 per cent w/v *diammonium hydrogen citrate* and 5 per cent w/v solution of *disodium edetate*. Add 0.2 ml of *thymol blue solution* and neutralise with *5 M ammonia*. Add 10 ml of *sodium diethyldithiocarbamate solution* and 15 ml of *carbon tetrachloride*, shake and allow to separate. The yellow colour of the *carbon tetrachloride* layer is not more intense than that produced by treating 2 ml of *copper standard solution (10 ppm Cu)* in the same manner beginning at the words "Transfer to a separating funnel using....." (20 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v each of *mefenamic acid impurity C* and *mefenamic acid impurity D* in the mobile phase.

Reference solution (c). A 0.001 per cent w/v solution of *mefenamic acid impurity A IPRS* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (d). A 0.002 per cent w/v solution of *benzoic acid* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 14 volumes of *tetrahydrofuran*, 40 volumes of a 0.58 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 5.0 with *dilute ammonia* and 46 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Mefenamic acid impurity C[1]	0.3	5.9
Mefenamic acid impurity D[2]	0.35	4.0
Mefenamic acid impurity A[3]	0.5	—
Mefenamic acid (Retention time: about 8 minutes)	1.0	—

[1]2-chlorobenzoic acid,

[2]benzoic acid,

[3]2,3-dimethylaniline.

Inject reference solution (b) and (d). The test is not valid unless the resolution between the peaks due to mefenamic acid impurities C and D is not less than 3.0 in the chromatogram obtained with reference solution (b) and the signal-to-noise ratio is not less than 10 for the principal peak in the chromatogram obtained with reference solution (d).

Inject reference solution (a), (c) and the test solution. Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to mefenamic acid impurities C and D is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of any peak due to mefenamic acid impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (100 ppm), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

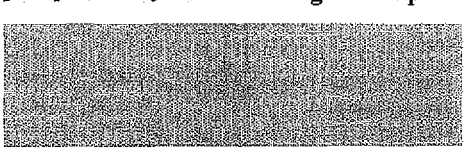
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.5 g and dissolve in 100 ml of warm *ethanol (95 per cent)* previously neutralised to *phenol red solution* and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02413 g of $C_{15}H_{15}NO_2$.

Storage. Store protected from light and moisture.



Mefenamic Acid Capsules

Mefenamic Acid Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mefenamic acid, $C_{15}H_{15}NO_2$.

Usual strength. 250 mg; 500 mg.

Identification

Extract a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with two quantities, each of 30 ml, of *ether*. Wash the combined extracts with *water* and evaporate to dryness on a water-bath. The residue, after drying at 105°, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mefenamic acid IPRS* or with the reference spectrum of mefenamic acid.

B. Dissolve 25 mg in 15 ml of *chloroform* and examine under ultraviolet light at 365 nm; the solution exhibits a strong greenish-yellow fluorescence. Carefully add 0.5 ml of a saturated solution of *trichloroacetic acid* drop wise and examine again under ultraviolet light at 365 nm; the solution does not exhibit fluorescence.

C. Dissolve 5 mg in 2 ml of *sulphuric acid* and add 0.05 ml of *0.0167 M potassium dichromate*; an intense blue colour is produced immediately which fades rapidly to brownish-green.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.05 M tris buffer* prepared by dissolving 60.5 g *tris(hydroxymethyl) aminomethane* in 6000 ml *water*, diluting to 10,000 ml with *water* and adjusted to pH 9.0 ± 0.05 with *phosphoric acid*. 100 g of *sodium lauryl sulphate* is dissolved in 6000 ml of the above solution and further mixed with the remaining quantity of the solution,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary.

Reference solution. A 0.02 per cent w/v solution of *mefenamic acid IPRS* in the dissolution medium.

Use the chromatographic system described under Assay.

Calculate the content of $C_{15}H_{15}NO_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{15}H_{15}NO_2$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *toluene*, 25 volumes of *dioxan* and 1 volume of *glacial acetic acid*.

Test solution. The supernatant liquid obtained by shaking a quantity of the contents of the capsules containing 0.25 g of Mefenamic Acid with a mixture of 7.5 ml of *chloroform* and 2.5 ml of *methanol*.

Reference solution. Dissolve 5.0 mg of the substance under examination in 100 ml of a mixture of 3 volumes of *chloroform* and 1 volume of *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air, expose to iodine vapour for 5 minutes and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

2,3-Dimethylaniline. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *toluene*, 25 volumes of *dioxan* and 1 volume of *18 M ammonia*.

Test solution. The supernatant liquid obtained in the test for Related substances.

Reference solution. A 0.00025 per cent w/v solution of *2, 3-dimethylaniline* in a mixture of 3 volumes of *chloroform* and 1 volume of *methanol*.

Apply to the plate 40 µl of each solution. After development, dry the plate in a current of warm air. Spray the plate with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for 30 minutes and immediately expose to nitrous fumes in a closed glass chamber for 15 minutes (the nitrous fumes may be generated by adding *dilute sulphuric acid* dropwise to a solution containing 10 per cent w/v of *sodium nitrite* and 3 per cent w/v of *potassium iodide*). Place the plate in a current of warm air for 15 minutes and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in *ethanol (95 per cent)*. If necessary, allow to dry and repeat the spraying. Any spot corresponding to 2,3-dimethylaniline in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the mixed contents of 20 capsules containing about 50 mg of Mefenamic acid in a 250.0-ml volumetric flask, add 5 ml of *tetrahydrofuran*, shake for 10 minutes with the aid of ultrasound, dilute to volume with the mobile phase and filter.

Reference solution. A solution containing 0.02 per cent w/v of *mefenamic acid IPRS* in the mobile phase.

NOTE—Protect the solutions from light.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 23 volumes of *acetonitrile*, 20 volumes of *0.05 M of monobasic ammonium phosphate* adjusted to a pH of 5.0 with *3 M ammonia* and 7 volumes of *tetrahydrofuran*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the mefenamic acid peak is not less than 8200 theoretical plates, the tailing factor is not more than 1.6 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{15}H_5NO_2$ in the capsules.

Mefenamic Acid and Dicyclomine Hydrochloride Tablets

Mefenamic Acid and Dicyclomine Hydrochloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of mefenamic acid, $C_{15}H_{15}NO_2$ and dicyclomine hydrochloride, $C_{19}H_{35}NO_2,HCl$.

Usual strengths. Mefenamic acid, 250 mg and Dicyclomine hydrochloride, 10 mg; Mefenamic acid, 250 mg and Dicyclomine hydrochloride, 20 mg; Mefenamic acid, 500 mg and Dicyclomine hydrochloride, 20 mg.

Identification

A. In the Assay for mefenamic acid, the test solution shows an absorption maxima and minima at the same wavelength as that of reference solution of mefenamic acid.

B. In the Assay for dicyclomine hydrochloride, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution of dicyclomine hydrochloride.

Tests

Dissolution (2.5.2).

For Mefenamic acid —

Apparatus No. 2 (Paddle),
Medium. 900 ml of a *tris buffer* prepared by dissolving 36.5 g of *tris(hydroxymethyl) aminomethane* in 6000 ml *water* and adjusted to pH 9.0 with *orthophosphoric acid*. Add 60.0 g of *sodium lauryl sulphate* in the above solution,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Test solution. Dilute 2.0 ml of the filtrate to 25.0 ml with the dissolution medium.

Reference solution. Weigh accurately 25.0 mg of *mefenamic acid IPRS* and transfer to a 50-ml volumetric flask, add 30.0 ml of *methanol* and sonicate to dissolve. Make up the volume with *methanol* and mix. Dilute further 2.0 ml of the solution to 50.0 ml with dissolution medium.

Measure the absorbance of the reference solution and test solution at the maximum at about 254 nm (2.4.7). Calculate the content of $C_{15}H_{15}NO_2$ in the medium.

Q. Not less than 70 per cent of the stated amount of $C_{15}H_{15}NO_2$.

For Dicyclomine hydrochloride —

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.01M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Solvent mixture. A mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*.

Test solution. Dilute 5.0 ml of the filtrate to 10.0 ml with the mobile phase.

Reference solution. Weigh accurately 25 mg of *dicyclomine hydrochloride IPRS* and transfer to a 25-ml volumetric flask and dilute with solvent mixture and sonicate. Dilute further 1.0 ml of the solution to 100.0 ml with mobile phase

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.5 with *sodium hydroxide* solution, 75 volumes of *acetonitrile* and filter,
- flow rate: 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{19}H_{35}NO_2,HCl$.

Q. Not less than 70 per cent of the stated amounts of $C_{19}H_{35}NO_2,HCl$.

Uniformity of content. Complies with the test stated under Tablets.

For Dicyclomine Hydrochloride — Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse one tablet in 80.0 ml of solvent mixture with the aid of ultrasound and dilute to 100.0 ml with the same solvent. Further dilute 4.0 ml of the solution to 10.0 ml with mobile phase.

Reference solution. Dissolve 25.0 mg of *dicyclomine hydrochloride IPRS* in 20 ml of solvent mixture and dilute to 25.0 ml with the same solvent. Dilute 1.0 ml of the solution to 25.0 ml with mobile phase and mix.

Inject the reference solution and test solution.

Calculate the content of $C_{19}H_{35}NO_2$,HCl in the tablet.

Other tests. Comply with the tests stated under tablets.

Assay.

For Mefenamic Acid —

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg Mefenamic acid in 70 ml of *methanol* with the aid of ultrasound and dilute to 100.0 ml with *methanol*. Dilute 2.0 ml of the solution to 50.0 ml with *methanol*.

Reference solution. A 0.002 per cent w/v solution of *mefenamic acid IPRS* in *methanol*.

Measure the absorbance of the reference solution and the test solution at 254 nm (2.4.7). Calculate the content of $C_{15}H_{15}NO_2$.

For Dicyclomine Hydrochloride — Determine by liquid chromatography (2.4.14).

Solvent mixture. 70 volumes of *acetonitrile* and 30 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg Dicyclomine Hydrochloride in to 50-ml volumetric flask add 40 ml of solvent mixture and sonicate to dissolve. Make up the volume with solvent mixture and mix. Further dilute 1.0 ml of the solution to 10.0 ml with mobile phase.

Reference solution. Weigh accurately 25 mg of *dicyclomine hydrochloride IPRS* and transfer to a 25-ml volumetric flask, add 20.0 ml of solvent mixture and sonicate to dissolve. Make up the volume with solvent mixture and mix. Dilute 1.0 ml of the solution to 25.0 ml with mobile phase and mix.

Chromatographic system

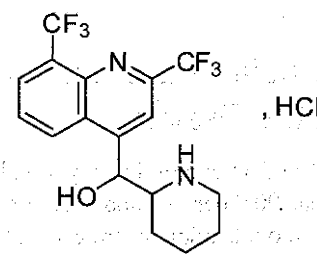
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* into 1000 ml of *water*, adjusted to pH 7.5 with *sodium hydroxide* solution, 75 volumes of *acetonitrile* and filter.
- flow rate: 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution and test solution.

Calculate the content of $C_{19}H_{35}NO_2$,HCl in the tablets.

Mefloquine Hydrochloride



$C_{17}H_{17}ClF_6N_2O$ Mol. Wt. 414.8

Mefloquine Hydrochloride is (*RS*)-[2,8-bis(trifluoromethyl) quinolin-4-yl][(2*SR*)-piperidin-2-yl]methanol hydrochloride.

Mefloquine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{17}ClF_6N_2O$ calculated on the anhydrous basis.

Category. Antimalarial.

Description. A white or slightly yellow crystalline powder.

Identification

Tests A and E may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mefloquine hydrochloride IPRS* or with the reference spectrum of mefloquine hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *anhydrous acetic acid*, 10 volumes of *methanol*, and 80 volumes of *dichloromethane*.

Test solution. Dissolve 8.0 mg of the substance under examination in 5.0 ml of *methanol*.

Reference solution (a). Dissolve 8.0 mg of *mefloquine hydrochloride IPRS* in 5.0 ml of *methanol*.

Reference solution (b). Dilute 2.5 ml of the test solution to 100 ml with *methanol*.

Reference solution (c). To 1 ml of reference solution (b), add 1 ml of a 0.0016 per cent w/v solution of *quinidine sulphate* in *methanol*.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air for 15 minutes and examine under ultraviolet light at 254 nm; lightly spray with a mixture, prepared immediately before use, of 1 volume of *sulphuric acid* and 40 volumes of *iodoplatinate reagent*; spray with *strong hydrogen peroxide solution*. In reference solution (c) the chromatogram shows two clearly separated spots. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solution (a).

C. Mix about 10 mg of the substance under examination with 45 mg of *heavy magnesium oxide* and ignite in a crucible until a practically white residue is obtained. Allow to cool, then add 2 ml of *water*, 0.05 ml of *phenolphthalein solution* and about 1 ml of *dilute hydrochloric acid* to make the solution colourless. Filter. To the filtrate add a freshly prepared mixture of 0.1 ml of *alizarin S solution* and 0.1 ml of *zirconyl nitrate solution*. Mix, allow to stand for 5 minutes and compare the colour of the solution with a blank prepared in the same manner. The test solution is yellow and the blank is red.

D. To about 20 mg of the substance under examination, add 0.2 ml of *sulphuric acid*. Blue fluorescence appears under ultraviolet light at 365 nm.

E. It gives reaction (B) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

Optical rotation (2.4.22). – 0.2° to + 0.2°, determined in a 5.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 25.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 20.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.016 per cent w/v each of *mefloquine hydrochloride IPRS* and *quinidine sulphate* in the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 100 mg of *tetraheptylammonium bromide* in a mixture of 20 volumes of *methanol*, 40 volumes of a 0.15 per cent w/v solution of *sodium hydrogen sulphate* and 40 volumes of *acetonitrile*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to quinidine and mefloquine is not less than 8.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of secondary peak with a relative retention time with reference to mefloquine is about 0.7 is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of secondary peaks other than the peak with a relative retention time with reference to mefloquine is about 0.7 is not more than five times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

Assay. Dissolve 0.35 g in 15 ml of *anhydrous formic acid* and add 40 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04148 g of $C_{17}H_{17}ClF_6N_2O$.

Storage. Store protected from light.

Mefloquine Tablets

Mefloquine Hydrochloride Tablets

Mefloquine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mefloquine, $C_{17}H_{16}F_6N_2O$.

Usual strength. 250 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 70 volumes of *toluene*, 30 volumes of *ethanol* and 2 volumes of *strong ammonia solution*.

Test solution. Disperse a quantity of powder containing about 0.1 g of Mefloquine in 10.0 ml of *methanol*.

Reference solution. A 1.0 per cent w/v solution of *mefloquine IPRS* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8.0 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted if necessary with the medium, at the maximum at about 283 nm (2.4.7). Calculate the content of $C_{17}H_{16}F_6N_2O$ in the medium from the absorbance obtained from a solution of known concentration of *mefloquine hydrochloride IPRS*, prepared by dissolving in minimum quantity of *methanol* and diluting with the dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{17}H_{16}F_6N_2O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the powdered tablets containing about 200 mg of Mefloquine, disperse in 100.0 ml of the mobile phase.

Reference solution (a). A solution containing 0.022 per cent w/v of *mefloquine hydrochloride IPRS* and 0.004 per cent w/v of *sulphadoxine* with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 2.0 ml of the solution to 20.0 ml with mobile phase.

Use chromatographic system as described in the Assay.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to mefloquine and sulphadoxine is not less than 5.0.The relative retention time with reference to mefloquine for (*RS*)-[2,8-bis(trifluoromethyl) quinolin-4-yl][(2*RS*)-piperidin-2-yl]methanol(threo-mefloquine impurity A) is about 0.9, for (*RS*)-[2,8-bis(trifluoromethyl) quinolin-4-yl](pyridin-2-yl]methanol (mefloquine impurity C) is about 3.6, for (*RS*)-[2,8-bis(trifluoromethyl)quinolin-4-yl](pyridin-2-yl]methanone (mefloquine impurity B) is about 7.4.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Disperse a quantity of the powdered tablets containing about 200 mg of mefloquine, disperse in 100.0 ml of the mobile phase.

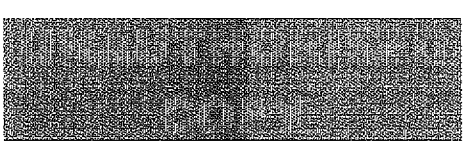
Test solution (b). Dilute 5.0 ml of test solution (a) to 50.0 ml with the mobile phase.

Reference solution (a). A 0.022 per cent w/v solution of *mefloquine hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.022 per cent w/v of *mefloquine hydrochloride IPRS* and 0.004 per cent w/v of *sulphadoxine* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 22 volumes of *methanol*, 38 volumes of *acetonitrile* and 40 volumes of a buffer solution prepared by dissolving 13.6 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *orthophoshphoric acid*,
- flow rate: 1.5 ml per minute,



– spectrophotometer set at 283 nm,
– injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to mefloquine and sulphadoxine is not less than 5.0.

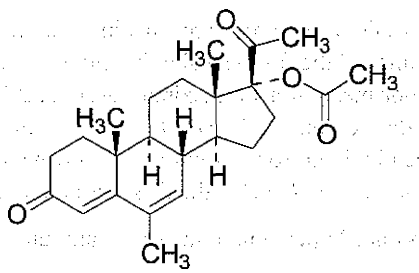
Inject reference solution (a) and test solution (b).

Calculate the content of $C_{17}H_{16}F_6N_2O$ in the tablets.

Each mg of mefloquine hydrochloride, $C_{17}H_{16}F_6N_2O,HCl$ is equivalent to 0.912 mg of mefloquine $C_{17}H_{16}F_6N_2O$.

Storage. Store protected from light and moisture.

Megestrol Acetate



$C_{24}H_{32}O_4$ Mol.Wt. 384.5

Megestrol Acetate is 6-methyl-3,20-dioxopregna-4,6-dien-17-yl acetate.

Megestrol Acetate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{24}H_{32}O_4$, calculated on the dried basis.

Category. Progestogen.

Description. A white to creamy-white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *megestrol acetate IPRS* or with the reference spectrum of megestrol acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 90 volumes of *acetone* and *1,2-propanediol*.

Mobile phase. A mixture of 40 volumes of *cyclohexane* and 10 volumes of *toluene*.

Test solution. Dissolve 25 mg of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *megestrol acetate IPRS* in 10.0 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 1µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. It gives the reaction of acetyl groups (2.3.1).

Tests

Specific optical rotation (2.4.22). +9.0° to +12.0°, determined at 20° in a 5.0 per cent w/v solution in *chloroform*.

Light absorption. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 287 nm; ratio of the absorbance at about 240 nm to that at the maximum at about 287 nm, not more than 0.17.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 145 volumes of *tetrahydrofuran* and 255 volumes of *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in 20 ml of the solvent mixture, dilute to 50.0 ml with *water*.

Reference solution (a). Dissolve 25 mg of *medroxyprogesterone acetate IPRS (megestrol acetate impurity A IPRS)* in 20 ml of the solvent mixture, dilute to 50.0 ml with *water*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 200.0 ml with the mobile phase.

Reference solution (c). To 3.0 ml of the test solution, add 1 ml of reference solution (a) and dilute to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 14.5 volumes of *tetrahydrofuran*, 22.5 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to megestrol acetate and megestrol acetate impurity A is not less than 4.0.

Inject reference solution (b) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak corresponding to megestrol acetate impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks other than megestrol acetate impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principle peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 20 mg in sufficient *ethanol (95 per cent)* to produce 100.0 ml, dilute 5.0 ml to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 287 nm (2.4.7). Calculate the content of $C_{24}H_{32}O_4$ taking 630 as the specific absorbance at 287 nm.

Storage. Store protected from light and moisture.

Megestrol Tablets

Megestrol AcetateTablets

Megestrol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of megestrol acetate, $C_{24}H_{32}O_4$.

Usual strengths. 20 mg; 160 mg.

Identification

A. Extract a quantity of the powdered tablets containing 40 mg of Megestrol Acetate with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness in a current of air. Dry the residue at 60° at a pressure not exceeding 0.7 kPa for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *megestrol acetate IPRS* or with the reference spectrum of megestrol acetate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 1 per cent w/v solution of *sodium lauryl sulphate*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably dilute if necessary with the dissolution medium, at the maximum at about 292 nm (2.4.7). Calculate the content of $C_{24}H_{32}O_4$ in the medium from the absorbance obtained from a solution of known concentration of *megestrol acetate IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{24}H_{32}O_4$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *acetonitrile* and 60 volumes of *water*

Internal standard solution. A 0.08 per cent w/v solution of *propylparaben* in *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 80 mg of Megestrol Acetate in 10 ml of *water*, shake for 10 minutes, add 75 ml of *acetonitrile*, shake for 30 minutes and dilute to 100.0 ml with *acetonitrile* and centrifuge. Dilute 5.0 ml of the solution and 5.0 ml of the internal standard solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *megestrol acetate IPRS* in *acetonitrile*. Dilute 4.0 ml of the solution and 5.0 ml of the internal standard solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *acetonitrile* and 45 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 25 µl.

The relative retention time with reference to megestrol acetate for propylparaben is about 0.4.

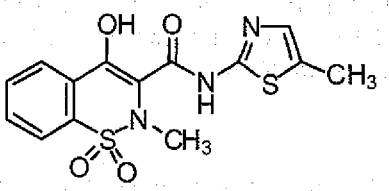
Inject the reference solution. The test is not valid unless the resolution between the peaks due to propylparaben and megestrol acetate is not less than 8.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for the peak response ratio of megestrol acetate and propyl paraben.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{32}O_4$ in the tablets.

Storage. Store protected from light and moisture.

Meloxicam



$C_{14}H_{13}N_3O_4S_2$ Mol. Wt. 351.4

Meloxicam is 4-Hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide.

Meloxicam contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{13}N_3O_4S_2$, calculated on the dried basis.

Category. Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Description. A pale yellow powder. It shows polymorphism (2.5.11).

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *meloxicam IPRS* or with the reference spectrum of meloxicam.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in a mixture of 5 ml of *methanol* and 0.3 ml of *1 M sodium hydroxide*. Dilute to 20.0 ml with *methanol*.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 5.0 ml of the solution to 100.0 ml with the same solvent.

Reference solution (b). Dissolve 2 mg of the substance under examination, 2 mg of *meloxicam impurity A IPRS*, 2 mg of *meloxicam impurity B IPRS*, 2 mg of *meloxicam impurity C IPRS* and 2 mg of *meloxicam impurity D IPRS* in a mixture of 5 ml of *methanol* and 0.3 ml of *1 M sodium hydroxide*. Dilute to 25.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: A. a 0.1 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 6.0 with *1 M sodium hydroxide*,
  B. *methanol*,
- flow rate: 1 ml per minute,
- a gradient programme using the conditions given below,
- spectrophotometer set at 260 nm and 350 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
2	60	40
10	30	70
15	30	70

Name	Relative retention time	Correction factor
Meloxicam impurity B[1]	0.5	---
Meloxicam (retention time is about 7 minutes)	1.0	---
Meloxicam impurity A[2]	1.4	2.0
Meloxicam impurity C[3]	1.7	---
Meloxicam impurity D[4]	1.9	---

[1]5-methylthiazol-2-amine.

[2]ethyl 4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide.

[3]*N*-[(2*Z*)-3,5-dimethylthiazol-2(3*H*)-ylidene]-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide.

[4]*N*-[(2*Z*)-3-ethyl-5-methylthiazol-2(3*H*)-ylidene]-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to meloxicam and meloxicam impurity A at 350 nm is not less than 3.0 and between the peaks due to meloxicam impurity B and meloxicam at 260 nm is not less than 3.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to meloxicam impurity A at 350 nm is not

more than the area of the principal peak in the chromatogram obtained with reference solution (a) at 350 nm (0.1 per cent), the area of any peak corresponding to meloxicam impurity B at 260 nm is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) at 350 nm (0.1 per cent), the area of any peak corresponding to meloxicam impurities C and D at 350 nm is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) at 350 nm (0.05 per cent), the area of any other secondary peak at 350 nm is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak at 350 nm with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.03 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. *To avoid overheating, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached.*

Dissolve 0.25 g in a mixture of 5 ml of *anhydrous formic acid* and 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03514 g of $C_{14}H_{13}N_3O_4S_2$.

Storage. Store protected from light

Meloxicam Oral Suspension

Meloxicam Oral Suspension is a suspension of Meloxicam in a suitable vehicle.

Meloxicam Oral Suspension contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of meloxicam, $C_{14}H_{13}N_3O_4S_2$.

Usual strengths. 7.5 mg; 15 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 1 volume of *13.5 M ammonia*, 20 volumes of *methanol* and 80 volumes of *dichloromethane*.

Test solution. Dilute a quantity of the oral suspension containing 3 mg of Meloxicam to 10 ml with *acetone*, stir for 10 minutes, filter and use the filtrate.

Reference solution. Dissolve 3 mg of *meloxicam IPRS* in about 5 ml of *acetone*, add 0.5 ml of *water* and dilute to 10 ml with *acetone*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine under ultraviolet light at 254 and 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Disperse a quantity of the substance under examination containing 1.5 mg of Meloxicam in 5 ml of *0.1M sodium hydroxide*, dilute to 100 ml with *methanol* and filter. The light absorption of the filtrate (2.4.7), in the range 340 to 450 nm exhibits a maximum at 362 nm.

Tests

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the oral suspension containing 15 mg of Meloxicam with sufficient of the mobile phase to produce 50 ml, stir for 30 minutes and filter.

Reference solution. A 0.03 per cent w/v solution of *meloxicam IPRS* in the mobile phase.

Chromatographic system

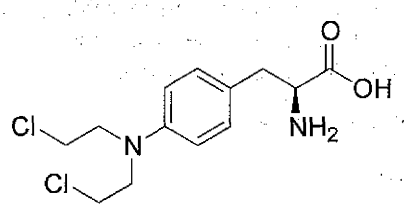
- a stainless steel column 10 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- column temperature: 40°,
- mobile phase: a mixture of 35 volumes of a solution containing 10 volumes of *propan-2-ol* and 65 volumes of *methanol* and 65 volumes of a 0.2 per cent w/v solution of *diammonium hydrogen orthophosphate* previously adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Determine the weight per ml of the suspension (2.4.29) and calculate the content of $C_{14}H_{13}N_3O_4S_2$.

Melphalan



$C_{13}H_{18}Cl_2N_2O_2$ Mol. Wt. 305.2

Melphalan is 4-bis(2-chloroethyl)amino-L-phenylalanine.

Melphalan contains not less than 93.0 per cent and not more than 100.5 per cent of $C_{13}H_{18}Cl_2N_2O_2$, calculated on the dried basis.

Category. Anticancer.

Description. A white or almost white powder.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 260 nm and a less well-defined maximum at about 301 nm.

B. Dissolve 20 mg in 50 ml of *methanol* with the aid of gentle heat, add 1 ml of a 5 per cent w/v solution of *4-(4-nitrobenzyl)pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 ml of hot *methanol* and add 0.1 ml of *strong ammonia solution*; a red colour is produced.

C. Heat 0.1 g with 10 ml of *0.1 M sodium hydroxide* for 10 minutes on a water-bath. The resulting solution, after acidification with *2 M nitric acid*, gives reaction (A) of chlorides (2.3.1).

Tests

Specific optical rotation (2.4.22). –36.0° to –30.0°, determined in a 0.7 per cent w/v solution in *methanol* prepared with the aid of gentle heat.

Ionisable chlorine. Dissolve 0.4 g in a mixture of 75 ml of *water* and 2 ml of *nitric acid*. Allow to stand for 2 minutes and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25); not more than 0.8 ml is required.

Sulphated Ash (2.3.18). Not more than 0.3 per cent.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 0.5 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa for 2 hours.

Assay. Weigh 0.4 g, add 20 ml of a 20 per cent w/v solution of *potassium hydroxide*, heat on a water-bath for 2 hours, replacing the *water* lost by evaporation. Cool, add 75 ml of *water* and 4 ml of *nitric acid*, cool. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25). Subtract the equivalent volume of *0.1 M silver nitrate* used in the test for Ionisable chlorine. The difference between the volumes represents the amount of *0.1 M silver nitrate* required by melphalan.

1 ml of *0.1 M silver nitrate* is equivalent to 0.01526 g of $C_{13}H_{18}Cl_2N_2O_2$.

Storage. Store protected from light and moisture.

Melphalan Injection

Melphalan Injection is a sterile material consisting of Melphalan with or without auxiliary substances. It is filled in a sealed container.

Usual strength. 50 mg per vial

The inection is prepared immediately before use by dissolving the contents of the sealed container which contains Melphalan Hydrochloride with or without auxillary substances in a suitable solvent and then diluting with the requisite volume of a suitable diluent in accordance with the manufacturer's instructions.

The constituted solution complies with the requirement for Particulate matter stated under Parenteral Preparations (Injections) and with the following tests.

Tests

Solution A. Dissolve the contents of one container in a suitable solvent, dilute the requisite volume of a suitable diluent in accordance with the manufacturer's instruction to produce a final solution containing 0.5 per cent w/v of anhydrous melphalan and allow to stand for 30 minutes.

Appearance of solution. Solution A is not more opalescent than opalescence standard OS2 (2.4.1).

pH (2.4.24). 6.0 to 7.0, determined in solution A.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Melphalan Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous melphalan, $C_{13}H_{18}Cl_2N_2O_2$.

Usual strength. 50 mg.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements:

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maxima at about 260 nm and a less well-defined maxima at about 301 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Dissolve a quantity containing 20 mg of anhydrous melphalan in 50 ml of *methanol* with the aid of gentle heat, add 1 ml of a 5 per cent w/v solution of *4-(4-nitrobenzyl)pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 ml of hot *methanol* and add 0.1 ml of *strong ammonia solution*; a red colour is produced.

D. Heat a quantity of the powder containing 0.1 g of anhydrous melphalan with 10 ml of *0.1 M sodium hydroxide* for 10 minutes on a water-bath. The resulting solution, after acidification with *2 M nitric acid*, gives reaction (A) of chlorides (2.3.1).

Tests

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 4 volumes of *acetonitrile* and 1 volume of *0.1 M hydrochloric acid*.

Test solution. Weigh a quantity of the mixed contents of 10 containers containing about 50 mg of Melphalan, dissolve in solvent mixture and dilute with sufficient of the solvent mixture to produce a final solution containing the equivalent of 0.01 per cent w/v of anhydrous melphalan.

Reference solution. A 0.01 per cent w/v solution of *melphalan IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (10 µm),
- mobile phase: 200 volumes of a 0.375 per cent w/v solution of *ammonium carbonate*, 180 volumes of *methanol* and 2.7 volumes of *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in the injection.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label on the sealed container states (1) the equivalent amount of anhydrous melphalan contained in it; (2) that it should be used immediately after preparation.

Melphalan Tablets

Melphalan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of melphalan, $C_{13}H_{18}Cl_2N_2O_2$. The tablets are coated.

Usual strengths. 2 mg; 4 mg.

Identification

A. Shake a quantity of the powdered tablets containing about 5 mg of Melphalan with 100 ml of hot *methanol*. Filter and dilute 10 ml of the filtrate to 50 ml with *methanol*.

When examined in the range 230 nm to 360 nm (2.4.7), the filtrate shows an absorption maxima at about 260 nm and a less well-defined maxima at about 301 nm.

B. To the remainder of the filtrate obtained in test A add 1 ml of a 5 per cent w/v solution of *4-(4-nitrobenzyl)pyridine* in *acetone* and evaporate to dryness. Dissolve the residue in 1 ml of hot *methanol* and 0.1 ml of *strong ammonia solution*; a red colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with *0.1 M hydrochloric acid*.

Reference solution. Dilute a suitable volume of 0.1 per cent w/v solution of *melphalan IPRS* in a mixture of 4 volumes of *acetonitrile* and 1 volume of *0.1 M hydrochloric acid*, with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.0002 per cent w/v.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as spherisorb ODS 1),
- mobile phase: a mixture of 200 volumes of 0.375 per cent w/v solution of *ammonium carbonate*, 180 volumes of *methanol* and 2.7 volumes of *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{13}H_{18}Cl_2N_2O_2$.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14)

Test solution. Add 20 ml of a mixture of 4 volumes of *acetonitrile* and 1 volume of *0.1 M hydrochloric acid* to one tablet, mix with the aid of ultrasound for 10 minutes or until the tablet disintegrates, filter, discarding the first 5 ml of filtrate, and use the filtrate.

Reference solution. A 0.01 per cent w/v solution of *melphalan IPRS* in the same solvent.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 200 volumes of a 0.375 per cent w/v solution of *ammonium carbonate*, 180 volumes of *methanol* and 2.7 volumes of *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets and add about 150 ml of a mixture of 4 volumes of *acetonitrile* and 1 volume of *0.1 M hydrochloric acid* to a weighed quantity of the powdered tablets containing about 25 mg of Melphalan, shake and mix with the aid of ultrasound for 5 minutes, dilute to 250 ml with the same solvent. Filter, discarding the first 20 ml of filtrate, and use the filtrate.

Reference solution. A 0.01 per cent w/v solution of *melphalan IPRS* in the same solvent.

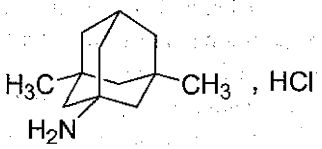
Use the chromatographic system described under Uniformity of content.

Calculate the content of $C_{13}H_{18}Cl_2N_2O_2$ in the tablets (for tablets containing more than 2 mg of Melphalan).

For tablets containing 2 mg or less of Melphalan, use the average of 10 individual results obtained in the test for Uniformity of content.

Storage. Store protected from light and moisture in a cool place.

Memantine Hydrochloride



$C_{12}H_{21}N,HCl$ Mol. Wt. 215.8

Memantine Hydrochloride is tricyclo [3.3.1.1^{3,7}] decan-1-amine, 3,5-dimethyl-hydrochloride;1-amino-3,5-dimethyl-adamantane hydrochloride.

Memantine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{21}N,HCl$, calculated on the anhydrous basis.

Category. Antialzheimer.

Description. A white to off white, coloured powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *memantine hydrochloride IPRS* or with the reference spectrum of memantine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Transfer 250 mg of the substance under examination to a 50-ml volumetric flask. Add about 7.5 ml of *5 M sodium hydroxide* and 10.0 ml of *n-hexane*. Shake for 10 minutes. Transfer this solution to a separator. Allow the layers to separate, filter the upper layer, dry over *anhydrous sodium sulphate*. Allow to stand for few minutes. Use the clear filtrate.

Reference solution (a). A solution containing 0.025 per cent w/v each of *memantine impurity A IPRS*, *memantine impurity B IPRS*, *memantine impurity C IPRS*, *memantine impurity D IPRS* and *memantine impurity E IPRS* in *n-hexane*.

Reference solution (b). To a 100-ml volumetric flask containing 0.5 g of *memantine hydrochloride IPRS*. Add 20 ml of *5 M sodium hydroxide* and 20.0 ml of *n-hexane*. Shake for 10 minutes. Transfer this solution to a separator. Allow the layers to separate, filter the upper layer, dry over *anhydrous*

sodium sulphate. Allow to stand for few minutes. Use the clear filtrate.

Reference solution (c). Dilute 1.0 ml each of reference solution (a) and reference solution (b) to 10.0 ml with *n-hexane*. This solution contains 0.0025 per cent w/v each of *memantine impurity A IPRS, memantine impurity B IPRS, memantine impurity C IPRS, memantine impurity D IPRS, memantine impurity E IPRS and 0.25 per cent w/v of memantine hydrochloride IPRS*. (*Memantine impurity D IPRS* and E are used for identification purpose only).

Reference solution (d). Dilute 1.0 ml of reference solution (b) to 10.0 ml with *n-hexane*. Further dilute 1.0 ml of the solution and 10.0 ml of reference solution (a) to 100.0 ml with *n-hexane*. This solution contains 0.0025 per cent w/v each of *memantine impurity A IPRS, memantine impurity B IPRS, memantine impurity C IPRS, memantine impurity D IPRS, memantine impurity E IPRS* and *memantine IPRS* in *n-hexane*.

Chromatographic system as described under Assay.

Name	Relative retention time
Memantine impurity A[1]	0.77
Memantine	1.0
Memantine impurity B[2]	1.03
Memantine impurity C[3]	1.07
Memantine impurity D[4]	1.19
Memantine impurity E[5]	1.44

[1]1,3-dimethyladamantane,

[2]3,5-dimethyladamantane-1-ol,

[3]1-chloro-3,5-dimethyladamantane,

[4]1-bromo-3,5-dimethyladamantane,

[5]3,5-dimethyladamantan-1-yl.

Ignore any peak with a relative retention time of 0.11, 0.12, 0.13, 0.18 and 0.26 with respect to memantine peak.

Inject reference solution (c) and (d). The test is not valid unless the resolution between peaks due to memantine and memantine impurity B is not less than 6 and the resolution between the peaks due memantine impurity B and memantine impurity C is not less than 2.0 obtained with reference solution (c). The tailing factor is not more than 2.0 for memantine peak obtained with reference solution (d).

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to memantine impurity A, B, C, D and E are not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Water (2.3.43). Not more than 1.0 per cent.

Assay. Determine by gas chromatography (2. 4.13).

Internal standard solution. A 0.4 per cent v/v solution of *adamantane* in *n-hexane*.

Test solution. Weigh and transfer 100 mg of the substance under examination, to a 60-ml separator. Add 15 ml of *1 M sodium hydroxide* and mix. Add 25.0 ml of internal standard solution, and shake for 15 minutes. Allow the layers to separate, and filter upper hexane layer through *anhydrous sodium sulphate*. Use the clear filtrate.

Reference solution. Weigh 100 mg of *memantine hydrochloride IPRS*, transfer to a 60-ml separator and dissolve in 15.0 ml of *1 M sodium hydroxide* and mix. Add 25.0 ml of internal standard solution, and shake for 15 minutes. Allow the layers to separate, and filter upper hexane layer through *anhydrous sodium sulphate*. Use the clear filtrate.

Chromatographic system

- a capillary column 50 m x 0.32 mm, coated with 5 per cent phenyl and 95 per cent methylpolysiloxane (film thickness 0.52 µm),
- temperature:
  column. 50°, then raised at the rate of 5° per minute upto 145° and then raised at the rate of 10° per minute up to 250° hold for 20 minutes,
- inlet port at 220° and detector at 300°,
- split ratio. 1:50,
- flame ionization detector,
- flow rate: 4.0 ml per minute, using helium as carrier gas,
- injection volume: 1 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 for memantine and adamantane peaks. The relative standard deviation of the peak response ratio replicate injections due to memantine and adamantane is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{12}H_{21}N,HCl$ from the peak response ratios of memantine to the internal standard obtained with the reference solution and the test solution respectively.

Storage: Store protected from light and moisture, at a temperature below 30°.

Memantine Tablets

Memantine Hydrochloride Tablets

Memantine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of memantine hydrochloride, $C_{12}H_{21}N,HCl$.

Usual strengths. 5 mg; 10 mg.

Identification

A. Extract a quantity of the powdered tablet containing 70 mg of memantine hydrochloride with 10 ml of *dichloromethane* and shaking for 10 minutes. Centrifuge for 10 minutes and use the supernatant liquid. Evaporate the solvent at room temperature. Collect the residue and dry at 60° for 15 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with 67 mg of *memantine hydrochloride IPRS* treated in the same manner or with the reference spectrum of memantine.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of *0.2 per cent w/v solution of sodium chloride* in *water*, adjusted to pH 1.2 with *hydrochloric acid*,

Speed and time. 100 rpm and 30 minutes.

Determine by gas chromatography (2.4.13).

Internal standard solution (a). 0.0028 per cent w/v solution of *amantadine hydrochloride IPRS* in medium.

Test solution. Withdraw a suitable volume of medium and filter. Transfer a volume of the filtrate, expected to contain 28 µg of memantine hydrochloride to a stoppered test tube, add 1.0 ml of the internal standard solution and 2 ml of *5 M sodium hydroxide*, and mix for 1 minute. Add 3.0 ml of *toluene*, and mix for 2 minutes. Use the toluene layer.

Reference solution (a). A solution containing 0.00056 per cent w/v solution of *memantine hydrochloride IPRS* in dissolution medium.

Reference solution (b). Transfer 5.0 ml of the reference solution (a) to a stoppered test tube, add 1.0 ml of the Internal standard solution and 2 ml of *5 M sodium hydroxide*, and mix for 1 minute. Add 3.0 ml of toluene, and mix for 2 minutes. Use the *toluene* layer.

Chromatographic system

- a capillary column 30 m x 0.32 mm, coated with 5 per cent phenyl and 95 per cent methylpolysiloxane (film thickness 0.25 µm),
- temperature:
  column. 50° for 2 minutes, then raised at the rate of 20° per minute upto at 140° and then raised at the rate of 30° per minute upto 200° and hold for 5 minutes,
- injection port at 210° and detector at 300°,
- flame ionization detector,
- flow rate: 7.0 ml per minute, using helium or nitrogen as carrier gas,
- injection volume: 4 µl,
- injection type: splitless.

Inject reference solution (b). The test in not valid unless the resolution between the peaks due to amantadine and memantine is not less than 2.0, the tailing factor due to amantadine and memantine peaks is not more than 2.0.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{12}H_{21}N,HCl$ the tablets from the peak response ratios of memantine to the amantadine obtained with reference solution (b) and the test solution respectively.

Q. Not less than 80 per cent of the stated amount of $C_{12}H_{21}N,HCl$.

Related substances. A. Determine by gas chromatography (2.4.13).

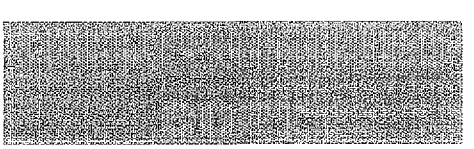
Test solution. Transfer a quantity of the powdered tablets containing 100 mg of Memantine Hydrochloride to a 100-ml volumetric flask. Add 15 ml of *1 M sodium hydroxide solution*. Shake for 5 minutes, mix with the aid of ultrasound for 5 minutes. Add 20.0 ml of *n-hexane* and shake for 10 minutes. Transfer the contents into a separator. Allow the layers to separate, and filter a portion of the top hexane layer through *anhydrous sodium sulphate*.

Reference solution (a). A solution containing 0.05 per cent w/v each of *memantine impurity A IPRS*, *memantine impurity B IPRS*, *memantine impurity C IPRS*, *memantine impurity D IPRS* and *memantine impurity E IPRS* in *n-hexane*.

Reference solution (b). Weigh and transfer 75 mg of *memantine hydrochloride IPRS* to a suitable volumetric flask, add 9 ml of *1 M sodium hydroxide solution* and 6 ml of the *n-hexane*, and mix for 10 minutes. Transfer the contents to a separator. Allow the layers to separate, and filter a portion of the top hexane layer through *anhydrous sodium sulphate*.

Reference solution (c). Pipette 4.0 ml of the n-hexane layer from reference solution (b) in to a 10-ml volumetric flask. Add 0.5 ml of reference solution (a) and dilute to volume with *n-hexane*.

Reference solution (d). Weigh and transfer 25 mg of *memantine hydrochloride IPRS* to a 50-ml volumetric flask. Add 15.0 ml of *1 M sodium hydroxide solution* mix for 5 minutes. Add 20.0 ml of *n-hexane* and shake for 10 minutes.



Transfer the contents into a separator. Allow the layers to separate, and filter a portion of the top *n-hexane* layer through *anhydrous sodium sulphate*.

Reference solution (e). Dilute 2.0 ml of the reference solution (d) to 100.0 ml with *n-hexane*.

Chromatographic system

- a capillary column 50 m x 0.32 mm, coated with 5 per cent phenyl and 95 per cent methylpolysiloxane (film thickness 0.52 µm),
- temperature:
  column. 50° for 2 minutes, then raised @ of 5° per minute to hold at 145° and then raised the temperature @ of 10° per minute to 250° and hold for 20 minutes,
- injection port at 220° and detector at 300°,
- split ratio: 1:20,
- flame ionization detector,
- flow rate: 4 ml per minute, using helium or nitrogen as carrier gas,
- injection volume: 3µl.

Name	Relative retention time
Memantine impurity A[1]	0.77
Memantine	1.0
Memantine impurity B[2]	1.03
Memantine impurity C[3]	1.1
Memantine impurity D[4]	1.2
Memantine impurity E[5]	1.4

[1]1,3-dimethyladamantane,this is a process impurity,

[2]3,5-dimethyladamantane-1-ol,this is a process impurity,

[3]1-chloro-3,5-dimethyladamantane,this is a process impurity,

[4]1-bromo-3,5-dimethyladamantane,this is a process impurity,

[5]3,5-dimethyladamantan-1-yl.

Inject reference solution (c) and (e) . The test is not valid unless the resolution between the peaks due to memantine and memantine impurity B and between memantine impurity B and memantine impurity C is not less than 2.0 obtained with reference solution (c) and the tailing factor is not more than 2.0 obtained with reference solution (e).

Inject reference solution (e) and the test solution. In the chromatogram obtained with the test solution, the area of peak corresponding to memantine impurity E is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.3 per cent). The area of any other secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (e) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.5 per cent) excluding memantine lactose adduct.

B. Determine by Liquid chromatography (2.4.14).

NOTE — Perform this test if lactose is present in the formulation.

Test solution. Weigh a quantity of powdered tablets containing 100 mg of Memantine Hydrochloride into a 20-ml volumetric flask. Add 10.0 ml of mobile phase, and sonicate for 30 minutes. Centrifuge, and pass a portion of the solution through a suitable filter of 0.45µm.

Reference solution. A 0.02 per cent w/v solution of *memantine hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 3.3 g of *monobasic potassium phosphate* and 2.3 g of *sodium 1-octane sulphonate* in 1000 ml of *water*, adjusted to pH 6.1 by 4 per cent w/v solution of *sodium hydroxide*, and 4 volumes of *methanol* and 26 volumes of *acetonitrile*,
- flow rate: 1.3 ml per minute,
- refractive index detector,
- injection volume: 50 µl.

Name	Relative retention time	Correction factor
Memantine lactose adduct	0.41	1.89
Memantine	1.0	---

Inject the reference solution. The test is not valid unless the tailing factor is not more than 3.5.

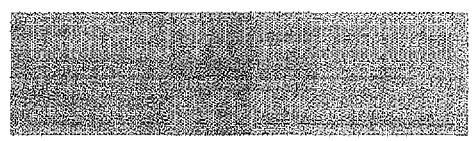
Inject the reference solution and the test solution. Run the chromatogram 1.3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to memantine lactose adduct is not more than 0.7 times the area of the principal peak in the chromatogram obtained with reference solution (1.4 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by gas chromatography (2.4.13), as described under Assay using the following solution

Test solution (a). Transfer one tablet to a 100-ml volumetric flask, add 40 ml of *water*; disperse with the aid of ultrasound for 30 minutes, further add 40 ml of *methanol* and shake for 10 minutes. Dilute to volume with *methanol* and centrifuge. Dilute a suitable volume of supernatant liquid with *water* to obtain a solution containing 0.002 per cent w/v of memantine hydrochloride.

Other tests. Comply with the tests stated under Tablets.



Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. A solution containing 0.0025 per cent w/v of *amantadine hydrochloride IPRS* in *water*.

Test solution (a). Weigh and powder 20 tablets. Disperse a quantity of the powder containing 10 mg of Memantine Hydrochloride in 40 ml of *methanol* and sonicate for 30 minutes, further add 40 ml of *water* and sonicate for 30 minutes with intermittent shaking. Dilute to 100.0 ml with *water*, and centrifuge a portion for 10 minutes. Dilute 2.0 ml of the clear, supernatant liquid to 10.0 ml with *water* and mix.

Test solution (b). Pipette 5.0 ml of the test solution (a) in to a stoppered test tube, add 4.0 ml of internal standard solution and add 2 ml of *20 per cent w/v solution of sodium hydroxide*, mix on a vortex mixer for 1 minute. Add 4.0 ml of *toluene*, and mix on a vortex mixer for 5 minutes. Allow the two layers to separate.

Reference solution (a). Dissolve 100 mg of *memantine hydrochloride IPRS* in *methanol* with the aid of ultrasound and dilute to 100.0 ml with *methanol*. Pipette 10.0 ml of the solution into a 100-ml volumetric flask, add 40 ml of *methanol* and dilute to volume with *water*. Dilute 2.0 ml of the solution to 10.0 ml with *water* and mix.

Reference solution (b). Pipette 5.0 ml of the reference solution (a) in to a stoppered test tube, add 4.0 ml of internal standard solution stoppered test tube, add 2 ml of 20 per cent w/v solution of *sodium hydroxide* and mix for 1 minute. Add 4.0 ml of *toluene*, and mix on a vortex mixer for 3 minutes. Allow the two layers to separate.

Blank. To 5.0 ml of 8.0 per cent v/v solution of *methanol* in *water* add 2 ml of 20 per cent w/v solution of *sodium hydroxide*, and mix for 1 minute. Add 4.0 ml of *toluene*, and mix on vortex for 3 minutes. Allow the two layers to separate. Inject the toluene layer.

Chromatographic system

- a capillary column 30 m x 0.32 mm, packed with 5 per cent phenyl and 95 per cent methylpolysiloxane (film thickness 0.25 µm),
- temperature:
  column. 50° for 2 minutes, then raised at the rate of 20° per minute to hold at 140° and then raised the rate of 30° per minute to 200° and hold for 5 minutes,
- injection port at 210° and detector at 300°,
- split ratio: 1:20,
- flame ionization detector,
- flow rate: 7 ml per minute, using helium or nitrogen as carrier gas,
- injection volume: 4 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to amantadine and memantine is not less than 2.0. The tailing factor is not more than 2.5 for amantadine and not more than 2.0 for memantine obtained from reference solution (b).

Inject the blank solution, reference solution (b) and test solution (b).

Calculate the content of $C_{12}H_{21}N$,HCl in the tablets from the peak response ratios of memantine to the amantadine obtained with reference solution (b) and test solution (b) respectively.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Menotropin

Human Menopausal Gonadotropin; Menotrophin

Menotropin is a sterile preparation containing glycoprotein gonadotropins possessing follicle stimulating and luteinizing activities. The preparation is exclusively or predominantly of pituitary origin and is extracted from the urine of post-menopausal women. Menotropin may be prepared by suitable fractionation procedure followed by ion-exchange chromatography. It is prepared in conditions designed to minimise microbial contaminations and to be in compliance with the requirements of viral safety.

Menotropin has the property in females of stimulating growth and maturation of ovarian follicles and in males of maintaining and stimulating testicular interstitial cells (Leydig tissue) related to testosterone production and of being responsible for full development and maturation of spermatozoa in seminiferous tubules.

Menotropin contains not less than 40 IU of Follicle Stimulating hormone (FSH) activity per mg. The ratio of IU of luteinizing hormone (LH) activity to IU of follicle stimulating hormone (FSH) is approximately 1. For each component the estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency.

Category. Gonadotropic hormone.

Description. An almost white or slightly yellow powder, soluble in *water*.

Identification

It causes enlargement of the ovaries of immature female rats and increases the weight of the seminal vesicles and prostate gland of immature male rats when administered as described under assay.

Tests

NOTE — Menotropin is prepared by suitable collection and extraction procedures followed by purification steps. The method of preparation includes steps that have been shown

to remove and / or inactivate extraneous agents including viral agents as determined by a suitable risk based approach as approved by the regulatory authority. The drug substance is negative for HIV, HCV and HBV using validated NAT (Nucleic Acid Test) based assays.

Hepatitis B surface antigen. Examine by a suitably sensitive immunochemical method (2.2.14). Hepatitis B surface antigens are not detected.

HCV antibodies. Examine by a suitably sensitive immunochemical method, (2.2.14) Hepatitis C antibodies are not detected.

HIV antibodies. Examine by a suitably sensitive immunochemical method, (2.2.14) HIV antibodies are not detected.

Water (2.3.43). Not more than 5.0 per cent w/w, by using 4 mg (Method 3).

Assay

Potency. The potency of menotropin with respect to its follicle stimulating hormone activity is estimated by comparing its effect in enlarging the ovaries of immature female rats with that of the standard preparation of human urinary FSH and human urinary LH under the conditions of suitable method of assay.

The potency of menotropin with respect to its luteinizing hormone activity is estimated by comparing its effect in increasing the weight of the seminal vesicles or the prostate gland of immature male rats with that of the standard preparation of urinary FSH and urinary LH under the conditions of suitable method of assay.

Standard preparation. The standard preparation is the International standard preparation of human urinary Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), consisting of freeze-dried extract from urine of post-menopausal together with lactose or any other suitable preparation, the potency of which has been determined in relation to the International reference standard.

a) Assay for Follicle stimulating hormone activity

Select female rats of the same strain, 19 to 28 days old and differing in age by not more than 3 days, and having weights such that the difference between the heaviest rat and the lightest rat is not more than 10 g. House the animals under uniform conditions of temperature, light, food and water. Mark the animals for identification, and divide them at random into seven equal groups of at least six animals per group. If sets of six litter mates are available, allot one litter mate from each set to each group and mark according to litter. Assign one group to each standard preparation one group to each assay preparation and one group to the control. Keep the control group during dose determination trail only.

Choose three doses of the standard preparation and three doses of the preparation being examined such that the smallest dose produces a positive response in some of the rats and the largest dose does not produce a maximum response in all of the rats. Use doses in geometric progression. As an initial approximation total doses of 1.5, 3.0, 6.0 IU may be tried although the dose will depend on the sensitivity of the animals used which may vary widely.

Standard solution. Dissolve a sufficient quantity of the standard preparation corresponding to the daily doses to be used in sufficient albumin-phosphate buffer pH 7.2 containing not less than 70 IU of Chorionic Gonadotropin per ml so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

Test solution. Prepare test solution similar to that of standard solution by dissolve a sufficient quantity of the preparation under examination instead of standard preparation.

Inject subcutaneously into each rat the daily dose allocated to its group. Repeat the procedure after 24 and 48 hours. About 24 hours after the last injection, euthanize the rats and remove the ovaries. Remove any extraneous fluid and tissue and immediately weigh the ovaries from each animal. Record the combined weight of both ovaries from each rat. Calculate the result of the assay by standard statistical methods using the weight of the ovaries as the response. (The precision of the assay may be improved by a suitable correction of the organ weight with reference to the weight of the animal from which it was taken; an analysis of covariance may be used). The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of stated potency.

b) Assay for Luteinizing hormone activity

Standard solution. Dissolve a sufficient quantity of standard preparation corresponding to the daily doses to be used in sufficient albumin-phosphate buffer pH 7.2 so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

Test solution. Prepare test solution similar to that of standard solution by dissolve a sufficient quantity of the preparation under examination instead of standard preparation.

Select male rats of the same strain, approximately 19 to 28 days old and differing in age by not more than 3 days, and having weights such that the difference between the heaviest rat and the lightest rat is not more than 10 g. House the animals under uniform conditions of temperature, light, food and water. Mark the animals for identification, and divide them at random into seven equal groups of at least six animals per group. If

sets of six litter mates are available, allot one litter mate from each set to each group and mark according to litter. Assign one group to each standard preparation one group to each assay preparation and one group to the control. Keep the control group during dose determination trail only.

Choose three doses of the Standard preparation and three doses of the preparation being examined such that the smallest dose is sufficient to produce a positive response in some of the rats and the largest dose does not produce a maximum response in all of the rats. Use doses in geometric progression. As an initial approximation, total doses of 7, 14 and 28 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely. Dissolve separately the total quantities of the preparation being examined and of the standard Preparation corresponding to the daily doses to be used in sufficient albumin phosphate buffer pH 7.2 so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solutions at a temperature of 2° to 8°, Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, euthanize the rats and remove the seminal vesicles or the prostate gland. Remove any extraneous fluid and tissue and weigh immediately the seminal vesicles or the prostate gland. Calculate the result of the assay by standard statistical methods, using the weight of the vesicles or the prostate gland as the response. (The precision of the assay may be improved by a suitable correction of the organ weight with reference to the weight of the animal from which it was taken; an analysis of covariance may be used). The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of stated potency.

Bacterial endotoxins (2.2.3). Not more than 0.78 IU per IU of Follicle-Stimulating Hormone.

Sterility (2.2.11). Complies with the test for sterility, if the menotropin intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity (2.2.1) using a quantity equivalent to 75 IU follicle stimulating hormone.

Storage. Menotropin should be kept in an airtight, tamper-evident container and protected from light. Store in a refrigerator at 2° to 8°.

Labelling. The label of the sealed container states (1) the number of IU (Units) of follicle-stimulating hormone activity, the number of IU (Units) of luteinizing hormone activity in the container; (2) The number of IU (Units) of follicle stimulating hormone activity per mg and the number of IU (units) of luteinizing hormone activity per mg; (3) where applicable, the number of IU (Units) of chorionic gonadotropin activity per mg; (4) the date after which the material is not intended to be used; (5) the condition under which it should be stored; (6) where applicable, that it is sterile.

Menotropin for Injection

Menotrophin for Injection

Menotropin for Injection is a sterile material consisting of menotropin with or without excipients. It may also contain an antimicrobial agent. It is supplied in a sealed container. Menotropin injection contains not less than 80 per cent and not more than 125 per cent of the stated potency.

The injection is reconstituted by dissolving the contents of the sealed container in the requisite amount of *Sterile Water for Injection* or a suitable diluent supplied by the manufacturer, immediately before use. The reconstituted solution should be used immediately after preparation but, in any case, within the period as recommended by the manufacturer.

The reconstituted solution complies with the requirements for Clarity of solution and Particulate matter stated under parenteral preparations (Injections).

Usual strengths. 75 IU per container, 150 IU per container.

Description. A white or almost white, slightly yellow powder, lyophilized powder

Identification

It causes enlargement of the ovaries of immature female rats and increases in the weight of the seminal vesicles and prostate gland of immature male rats when administered as described under the Assay.

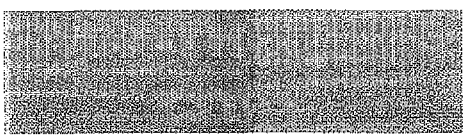
Tests

NOTE — Tests for Hepatitis B Surface antigen, HCV antibodies and HIV antibodies may be omitted if the menotropin for injection is prepared from menotropin complied as per monograph in current edition of IP. If any excipient of human origin isused, the injection must be free from HIV, HCV and HBV, confirmed by using validated NAT (Nucleic Acid Test) based assays.

pH (2.4.24). 6.0 to 8.0, dissolve the vial contents in 3ml of *water*.

Hepatitis B surface antigen. Examine by a suitably sensitive immunochemical method, (2.2.14) Hepatitis B surface antigens are not detected.

HCV antibodies. Examine by a suitably sensitive immunochemical method, (2.2.14) Hepatitis C antibodies are not detected.



HIV antibodies. Examine by a suitably sensitive immunochemical method, (2.2.14) HIV antibodies are not detected.

Water (2.3.43). Not more than 5.0 per cent (Method 3).

Other tests. Comply with the requirements stated under Powders for injections with the following requirements.

Bacterial endotoxins (2.2.3). Not more than 0.78 IU per IU of follicle stimulating hormone.

Assay

Potency. The potency of menotropin with respect to its follicle stimulating hormone activity is estimated by comparing its effect in enlarging the ovaries of immature female rats with that of the standard preparation of human urinary FSH and human urinary LH under the conditions of suitable method of assay.

The potency of menotropin with respect to its luteinizing hormone activity is estimated by comparing its effect in increasing the weight of the seminal vesicles or the prostate gland of immature male rats with that of the standard preparation of urinary FSH and urinary LH under the conditions of suitable method of assay.

Standard preparation. The standard preparation is the International standard preparation of human urinary Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), consisting of freeze-dried extract from urine of post-menopausal together with lactose or any other suitable preparation, the potency of which has been determined in relation to the International reference standard.

a) Assay for Follicle stimulating hormone activity

Select female rats of the same strain, 19 to 28 days old and differing in age by not more than 3 days, and having weights such that the difference between the heaviest rat and the lightest rat is not more than 10 g. House the animals under uniform conditions of temperature, light, food and water. Mark the animals for identification, and divide them at random into seven equal groups of at least six animals per group. If sets of six litter mates are available, allot one litter mate from each set to each group and mark according to litter. Assign one group to each standard preparation one group to each assay preparation and one group to the control. Keep the control group during dose determination trail only.

Choose three doses of the standard preparation and three doses of the preparation being examined such that the smallest dose produces a positive response in some of the rats and the largest dose does not produce a maximum response in all of the rats. Use doses in geometric progression. As an initial approximation total doses of 1.5, 3.0, 6.0 IU may be tried although the dose will depend on the sensitivity of the animals used which may vary widely.

Standard solution. Dissolve a sufficient quantity of the standard preparation corresponding to the daily doses to be used in sufficient albumin-phosphate buffer pH 7.2 containing not less than 70 IU of Chorionic Gonadotropin per ml so that the daily dose is about 0.2 ml.. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

Test solution. Prepare test solution similar to that of standard solution by dissolve a sufficient quantity of the preparation under examination instead of standard preparation.

Inject subcutaneously into each rat the daily dose allocated to its group. Repeat the procedure after 24 and 48 hours. About 24 hours after the last injection, euthanize the rats and remove the ovaries. Remove any extraneous fluid and tissue and immediately weigh the ovaries from each animal. Record the combined weight of both ovaries from each rat. Calculate the result of the assay by standard statistical methods using the weight of the ovaries as the response. (The precision of the assay may be improved by a suitable correction of the organ weight with reference to the weight of the animal from which it was taken; an analysis of covariance may be used). The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of stated potency.

b) Assay for Luteinizing hormone activity

Standard solution. Dissolve a sufficient quantity of standard preparation corresponding to the daily doses to be used in sufficient albumin-phosphate buffer pH 7.2 so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solution at a temperature of 2° to 8°.

Test solution. Prepare test solution similar to that of standard solution by dissolve a sufficient quantity of the preparation under examination instead of standard preparation.

Select male rats of the same strain, approximately 19 to 28 days old and differing in age by not more than 3 days, and having weights such that the difference between the heaviest rat and the lightest rat is not more than 10 g. House the animals under uniform conditions of temperature, light, food and water. Mark the animals for identification, and divide them at random into seven equal groups of at least six animals per group. If sets of six litter mates are available, allot one litter mate from each set to each group and mark according to litter. Assign one group to each standard preparation one group to each assay preparation and one group to the control. Keep the control group during dose determination trail only.

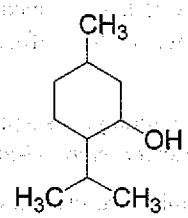
Choose three doses of the Standard preparation and three doses of the preparation being examined such that the smallest dose is sufficient to produce a positive response in some of the rats and the largest dose does not produce a maximum

response in all of the rats. Use doses in geometric progression. As an initial approximation, total doses of 7, 14 and 28 IU may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely. Dissolve separately the total quantities of the preparation being examined and of the standard Preparation corresponding to the daily doses to be used in sufficient albumin phosphate buffer pH 7.2 so that the daily dose is about 0.2 ml. Add a suitable antimicrobial preservative such as 0.4 per cent w/v of *phenol* or 0.002 per cent w/v of *thiomersal*. Store the solutions at a temperature of 2° to 8°, Inject subcutaneously into each rat the daily dose allocated to its group on 4 consecutive days at the same time each day. On the fifth day, about 24 hours after the last injection, euthanize the rats and remove the seminal vesicles or the prostate gland. Remove any extraneous fluid and tissue and weigh immediately the seminal vesicles or the prostate gland. Calculate the result of the assay by standard statistical methods, using the weight of the vesicles or the prostate gland as the response. (The precision of the assay may be improved by a suitable correction of the organ weight with reference to the weight of the animal from which it was taken; an analysis of covariance may be used). The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of stated potency.

Storage. Sealed container, store protected from light in containers at a temperature not exceeding 20°.

Labelling. The label states (1) the number of IU (units) of follicle stimulating hormone activity; (2) the number of IU (units) of luteinizing hormone activity and (3) where applicable, the number of IU (units) of chorionic gonadotrophin activity contained in it.

Menthol



$C_{10}H_{20}O$ Mol. Wt.156.3

Menthol is 2-isopropyl-5-methylcyclohexanol. It is obtained from the volatile oils of various species of *Mentha* or prepared synthetically. It may be levo-rotatory [(–)-menthol] or racemic [(±)-menthol].

Category. Topical antipruritic.

Description. Colourless, hexagonal or needle-like crystals, or infused masses or a crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *menthol IPRS* or with the reference spectrum of menthol.

B. Dissolve 10 mg in 1 ml of *sulphuric acid* and add 1 ml of a 1 per cent w/v solution of *vanillin* in *sulphuric acid*; an orange-yellow colour is produced. Add 1 ml of *water*; the colour changes to violet (distinction from thymol).

C. When triturated with about an equal weight of *camphor* or *chloral hydrate* or *phenol*, the mixture liquefies.

Tests

Appearance of solution. Dissolve 1.0 g in 10 ml of *ethanol (95 per cent)*. The solution is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution RS6 (2.4.1).

Acidity. To 1.0 g in a 100-ml glass-stoppered conical flask add 20 ml of *water*, boil until dissolution is complete, cool, stopper the flask and shake vigorously for 1 minute. Add a few crystals of the substance under examination to initiate crystallisation, shake vigorously for 1 minute and filter. To 5 ml of the filtrate add 0.05 ml of *methyl red solution* and 0.05 ml of *0.01M sodium hydroxide*; the solution is yellow.

Specific optical rotation (2.4.22). (for (–)-menthol) –51.0° to –49.0°; (for (±)-menthol) –2.0 ° to +2.0°, determined in a 10.0 per cent w/v solution in *ethanol (95 per cent)*.

Congealing range (2.4.10). (for (±)-menthol) 27.0° to 28.0°; on prolonged stirring, the temperature rises 30° to 32°.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.1 g in sufficient *ethanol (95 per cent)* to produce 10 ml.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with *ethanol (95 per cent)*.

Reference solution (b). Dilute 1 ml of reference solution (a) to 20 ml with *ethanol (95 per cent)*.

Chromatographic system

- a glass or stainless steel column 4 m x 2 mm, packed with diatomaceous support (125 to 180 mesh) impregnated with 5 per cent carbowax 20 M (Polyethylene glycol compund 20 M),
- temperature :
  column 80°, after 2 minutes, increase the temperature of the column to 240° at a rate of 8° per minute and maintain at this temperature for 15 minutes,
  injection port at 250° and the detector at 240°,
- flow rate: 30 ml per minute of the carrier gas.

Inject 1 µl of each solution. Run the chromatogram obtained with the test solution for 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Residue on evaporation. Evaporate 2.0 g on a water-bath and heat at 105° for 1 hour. The residue weighs not more than 1.0 mg (0.05 per cent).

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states whether the contents are levo-rotatory or racemic menthol.

Menthol and Benzoin Inhalation

Menthol and Benzoin Inhalation is an inhalation vapour, solution of racementhol or levomenthol 20 g in sufficient benzoin inhalation to produce 1000 ml.

Menthol and Benzoin Inhalation contains not less than 2.8 per cent w/v of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

Tests

Total solids (2.6.5). 9.0 per cent to 12.0 per cent w/v, determined on 2 ml of the solution by drying at 105° for 4 hours.

Other tests. Comply with the tests stated under Inhalation Preparations.

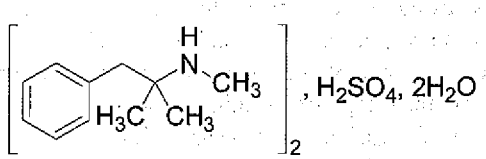
Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Boil 10 ml with 25 ml of *ethanolic potassium hydroxide solution* under a reflux condenser for 1 hour. Evaporate the *ethanol (95 per cent)*, disperse the residue in 50 ml of hot *water*, cool, add 80 ml of *water* and 1.5 g of *magnesium sulphate* dissolved in 50 ml of *water*. Mix thoroughly and allow to stand for 10 minutes. Filter, wash the residue on the filter with 20 ml of *water*, acidify the combined filtrate and washings with *hydrochloric acid* and extract with four 40 ml quantities of *ether*. Discard the aqueous solution, combine the ether extracts and extract with successive quantities of 20, 20, 10, 10 and 10 ml of *sodium hydrogen carbonate solution*, washing each aqueous extract with the same 20 ml of *ether*. Discard the ether layers, carefully acidify the combined aqueous extracts with *hydrochloric acid* and extract with successive quantities of 30, 20, 20 and 10 ml of *chloroform*, filtering each extract through *anhydrous sodium sulphate* supported on absorbent cotton. Distil the *chloroform* from the combined filtrates until 10 ml remains and remove the remainder in a current of air. Dissolve the residue, with the aid of gentle heat, in 10 ml of *ethanol (95 per cent)*, previously neutralised to *phenol red solution*, cool and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01482 g of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

Labelling. The label states the amount of active ingredient delivered per inhalation.

Mephentermine Sulphate



$(C_{11}H_{17}N)_2,H_2SO_4,2H_2O$ Mol. Wt.460.6

Mephentermine Sulphate is *N*,α,α-trimethylphenethylamine sulphate dihydrate.

Mephentermine Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{11}H_{17}N)_2,H_2SO_4$, calculated on the dried basis.

Category. Sympathomimetic.

Description. A white crystals or a white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mephentermine sulphate IPRS* or with the reference spectrum of mephentermine sulphate.

B. A 0.2 per cent w/v solution yields a precipitate with *iodine solution* and with *potassium mercuri-iodide solution*.

C. Dissolve 0.1 g in 5 ml of *water*, add with stirring 10 ml of *picric acid solution*. Allow to stand for 30 minutes, filter and wash the precipitate with small quantities of cold *water* until the last washing is colourless; the precipitate, after drying at 105° melts at 154° to 158° (2.4.21).

D. It gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.5, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 5.0 to 8.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

Assay. Weigh 0.4 g, dissolve in 250 ml of *water*, add 5 g of *sodium chloride*, shake well and add 5 ml of *5 M sodium hydroxide*. Extract with 30 ml and then with further quantities, each of 20 ml, of *ether* until the base is completely extracted. Combine the ether extracts, wash with two quantities, each of 10 ml, of *water* and extract the aqueous washings with 10 ml of *ether*, adding this ether to the main ether extract. Add to the ether solution 30.0 ml of *0.05 M sulphuric acid*, stir thoroughly and warm gently until the ether is evaporated. Cool and titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sulphuric acid required.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.02123 g of $(C_{11}H_{17}N)_2,H_2SO_4$.

Storage. Store protected from light and moisture.

Mephentermine Injection

Mephentermine Sulphate Injection

Mephentermine Injection is a sterile solution of Mephentermine Sulphate in Water for Injections.

Mephentermine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mephentermine, $C_{11}H_{17}N$.

Usual strength. The equivalent of 15 mg of mephentermine per ml (21 mg of mephentermine sulphate is approximately equivalent to 15 mg of mephentermine).

Identification

A. A 0.2 per cent w/v solution yields a precipitate with *iodine solution* and with *potassium mercuri-iodide solution*.

B. Dissolve 0.1 g in 5 ml of *water*, add with stirring 10 ml of *picric acid solution*. Allow to stand for 30 minutes, filter and wash the precipitate with small quantities of cold *water* until the last washing is colourless; the precipitate, after drying at 105° melts at 154° to 158° (2.4.21).

C. It gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.5.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Measure a volume containing about 0.2 g of mephentermine, add *water* if necessary to produce 20 ml add 5 g of *sodium chloride*, shake well and add 5 ml of *5 M sodium hydroxide*. Extract with 30 ml and then with further quantities, each of 20 ml, of *ether* until the base is completely extracted. Combine the ether extracts, wash with two quantities, each of 10 ml, of *water* and extract the aqueous washings with 10 ml of *ether*, adding this ether to the main ether extract. Add to the ether solution 30.0 ml of *0.05 M sulphuric acid*, stir thoroughly and warm gently until the ether is evaporated. Cool and titrate with *0.1 M sodium hydroxide* using *methyl red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sulphuric acid required.

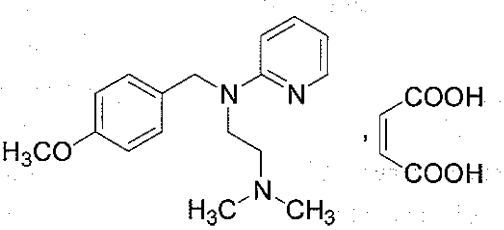
1 ml of *0.05 M sulphuric acid* is equivalent to 0.0163 g of $C_{11}H_{17}N$.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of mephentermine in a suitable dose-volume.

Mepyramine Maleate

Pyrilamine Maleate



$C_{17}H_{23}N_3O,C_4H_4O_4$ Mol. Wt. 401.5

Mepyramine Maleate is 2-(*N*-4-methoxybenzyl-*N*-2-pyridylamino)ethyldimethylamine hydrogen maleate.

Mepyramine Maleate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{23}N_3O,C_4H_4O_4$, calculated on the dried basis.

Category. Histamine H_1-receptor antagonist.

Description. A white or slightly yellowish, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mepyramine maleate IPRS* or with the reference spectrum of mepyramine maleate.

Tests

Appearance of solution. A 4.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 4.9 to 5.2, determined in a 2.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.0001 per cent w/v each of *anisaldehyde*, *mepyramine impurity A IPRS* and *mepyramine impurity C IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.1 volume of *triethylamine*, 40 volumes of a 0.08 per cent w/v solution of *ammonium acetate* and 60 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Name	Relative retention time
Maleic acid	0.2
Mepyramine impurity C[1]	0.3
Mepyramine impurity B[2]	0.4
Mepyramine impurity A[3]	0.5
Mepyramine (Retention time: about13 minutes)	1.0

[1]pyridin-2-amine,

[2]anisaldehyde,

[3]*N*-(4-methoxybenzyl)pyridin-2-amine.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to mepyramine impurities C and B is not less than 3.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to mepyramine impurities A and C is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent). The area of any peak corresponding to mepyramine impurity B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) and the peak due to maleic acid.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Chlorides (2.3.12). 2.5 g dissolved in 15 ml of *water* complies with the limit test for chlorides (100 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.25 per cent, determined on 2.0 g by drying in an oven at 80°.

Assay. Weigh 0.15 g, dissolve in 40 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02007 g of $C_{17}H_{23}N_3O,C_4H_4O_4$.

Storage. Store protected from light and moisture.

Mepyramine Tablets

Mepyramine MaleateTablets; Pyrilamine Maleate Tablets; Pyrilamine Tablets

Mepyramine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mepyramine maleate, $C_{17}H_{23}N_3O,C_4H_4O_4$.

Usual strength. 50 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Mepyramine Maleate with 10 ml of *dichloromethane*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mepyramine maleate IPRS* or with the reference spectrum of mepyramine maleate.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve a quantity of the powdered tablets containing 0.2 g of Mepyramine Maleate, freed as far as possible from any sugar coating, in 3 ml of *water*, add 2 ml of *5 M sodium hydroxide* and shake with three quantities, each of 3 ml, of *ether*. Warm the aqueous layer in a water-bath for 10 minutes with 2 ml of *bromine solution*, heat to boiling, cool and add 0.2 ml to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*; a bluish black colour is produced on heating for 15 minutes in a water-bath.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 100 volumes of *ethyl acetate* and 2 volumes of *diethylamine*.

NOTE—Prepare the following solutions immediately before use.

Test solution (a). Shake a quantity of the powdered tablets containing 0.4 g of Mepyramine Maleate with 10 ml of *chloroform* and filter.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *chloroform*.

Reference solution (a). A 4.0 per cent w/v solution of *mepyramine maleate IPRS* in *chloroform*.

Reference solution (b). A 0.4 per cent w/v solution of *mepyramine maleate IPRS* in *chloroform*.

Reference solution (c). A 0.008 per cent w/v solution of *mepyramine maleate IPRS* in *chloroform*.

Reference solution (d). A 0.004 per cent w/v solution of *mepyramine maleate IPRS* in *chloroform*.

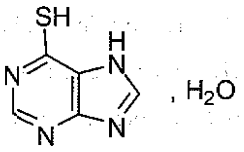
Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c). The test is not valid unless the R_f values of the principal spots in the chromatograms obtained with test solution (a) and reference solution (a) are at least 0.2 and unless the spot in the chromatogram obtained with reference solution (d) is clearly visible. Ignore the spot due to maleic acid on the line of application.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of Mepyramine Maleate, add 75 ml of *water* and 5 ml of *2 M hydrochloric acid*, shake vigorously for 15 minutes and dilute to 100.0 ml with *water*. Centrifuge and dilute 10.0 ml of the clear, supernatant liquid to 100.0 ml with *water*. To 10.0 ml add 10 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 316 nm (2.4.7). Calculate the content of $C_{17}H_{23}N_3O,C_4H_4O_4$ taking 206 as the specific absorbance at 316 nm.

Storage. Store protected from light and moisture.

Mercaptopurine



$C_5H_4N_4S,H_2O$ Mol. Wt. 170.2

Mercaptopurine is purine-6-thiol monohydrate.

Mercaptopurine contains not less than 98.5 per cent and not more than 101.0 per cent of $C_5H_4N_4S$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A yellow, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mercaptopurine IPRS* or with the reference spectrum of mercaptopurine.

B. Dissolve 20 mg in 5 ml of *dimethyl sulphoxide* and add sufficient *0.1 M hydrochloric acid* to produce 100 ml. Dilute 5 ml to 200 ml with *0.1 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 325 nm.

C. Dissolve 20 mg in 20 ml of *ethanol (95 per cent)* heated to 60° and add 1 ml of a saturated solution of *mercuric acetate* in *ethanol (95 per cent)*; a white precipitate is produced.

D. Dissolve 20 mg in 20 ml of *ethanol (95 per cent)* heated at 60° and add 1 ml of a 1 per cent w/v solution of *lead acetate* in *ethanol (95 per cent)*; a yellow precipitate is produced.

Tests

Hypoxanthine. Determine by thin layer chromatography (2.4.17), coating the plate with the *silica gel GF254*.

Mobile phase. A mixture of 3 volumes of *concentrated ammonia*, 7 volumes of *water* and 90 volumes of *acetone*.

Test solution. Dissolve 50 mg of the substance under examination in 1 ml of *dimethyl sulphoxide* and dilute to 10 ml with *methanol.*

Reference solution. Dilute 10 mg of *hypoxanthine* in 10 ml of *dimethyl sulphoxide* and diluted to 100 ml with *methanol.*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. After development, dry the plate in air and examine at 254 nm. Any secondary spot corresponding to hypoxanthine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (2.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 10.0 to 12.0 per cent, determined on 0.25 g.

Assay. Weigh 0.15 g, dissolve in 50 ml of *dimethylformamide.* Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.01522 g of $C_5H_4N_4S$.

Storage. Store protected from light and moisture.

Mercaptopurine Tablets

Mercaptopurine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mercaptopurine, $C_5H_4N_4S,H_2O$.

Usual strength. 50 mg.

Identification

Shake a quantity of the powdered tablets containing 50 mg of Mercaptopurine with a mixture of 20 ml of *water* and 0.5 ml of *5 M sodium hydroxide* for about 3 minutes, add sufficient *water* to produce 100 ml, mix and filter. Dilute a suitable aliquot of the filtrate with sufficient *0.1 M hydrochloric acid* to give a solution containing 5 µg of Mercaptopurine per ml. The resulting solution shows an absorption maximum at about 325 nm (2.4.7).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate as the test solution.

Reference solution. A solution containing 0.0055 per cent w/v of *mercaptopurine IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: 0.1 per cent v/v solution of *acetic acid* in *water*,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the retention time for mercaptopurine is not less than 4 minutes and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_5H_4N_4S,H_2O$ in the medium.

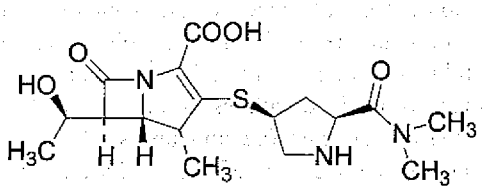
Q. Not less than 75 per cent of the stated amount of $C_5H_4N_4S,H_2O$.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 50 mg of Mercaptopurine, dissolve as completely as possible in 5 ml of *dimethyl sulphoxide* and add sufficient *0.1 M hydrochloric acid* to produce 500.0 ml. Dilute 5.0 ml to 100.0 ml with *0.1 M hydrochloric acid*, filter if necessary and measure the absorbance of the resulting solution at the maximum at about 325 nm (2.4.7). Calculate the content of $C_5H_4N_4S,H_2O$ taking 1165 as the specific absorbance at 325 nm.

Storage. Store protected from light and moisture.

Meropenem



$C_{17}H_{25}N_3O_5S,3H_2O$ Mol. Wt. 437.5 (hydrated)
$C_{17}H_{25}N_3O_5S$ 383.5 (anhydrous)

Meropenam is (1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-(dimethylaminocarbonyl)pyrrolidin-3-ylthio-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-en-3-carboxylic acid.

Meropenem contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{17}H_{25}N_3O_5S$, calculated on the anhydrous basis.

Category. Antibiotic.

Description. A white to off-white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *meropenem IPRS* or with the reference spectrum of meropenem.

Tests

pH (2.4.24). 4.0 to 6.0, determined in 1.0 per cent w/v solution in *water*.

Specific optical rotation (2.4.22). –21.0° to–17.0°, determined in a 0.5 per cent w/v solution.

Acetone (5.4). Not more than 0.05 per cent.

Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.005 per cent w/v solution of *ethyl acetate* in *dimethylformamide*.

Test solution. Dissolve 100 mg of the substance under examination in 0.2 ml of *dimethylformamide* and 2.0 ml of internal standard solution.

Reference solution. Weigh 50 mg of *acetone*, dissolve in a small quantity of *dimethylformamide*, dilute to 100.0 ml with *dimethylformamide* and mix. To 1.0 ml of the solution, add 10.0 ml of the internal standard solution, and mix.

Chromatographic system
- a glass column 2 m × 3 mm, packed with styrenedivinylbenzene copolymer (Such as Chromosorb 101),
- temperature:
  column. 150°,
  inlet port and detector 170°,
- flow rate adjusted so that the retention time for acetone is about 3 minutes of the carrier gas.

Inject 1 µl of the test solution and the reference solution.

Calculate the percentage of acetone.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. To 900 ml of *water* add 1.0 ml of *triethylamine*, adjusted to pH 5.0 with *dilute phosphoric acid*, dilute to 1000 ml with *water* and mix.

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 0.5 g of the substance under examination in the solvent mixture and dilute to 100 ml of the solvent mixture.

Reference solution. A 0.0025 per cent w/v solution of *meropenem IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: mix 1.0 ml of *triethylamine* and 900 ml of *water*, adjusted to pH 5.0 with *dilute orthophosphoric acid*, dilute with *water* to 1000 ml, add 70 ml of *acetonitrile*,
- flow rate: 1.6 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency in not less than 2500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all impurities found is not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1per cent, igniting at 500 ± 50°, instead of at 800 ± 25°. Use a desiccator containing *silica gel*.

Water (2.3.43). 11.4 per cent to 13.4 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. To 900 ml of *water* add 1.0 ml of *triethylamine*, adjusted to pH 5.0 with *dilute orthophosphoric acid*, dilute to 1000 ml with *water* and mix.

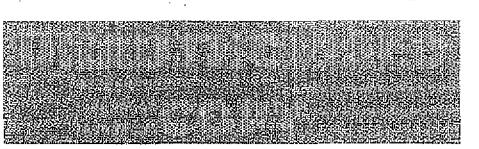
NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 50.0 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.05 per cent w/v solution of *meropenem IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 30°,
- mobile phase: a mixture of 50 volumes of the solvent mixture and 10 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 5 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency is not less than 2500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.



Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{25}N_3O_5S$.

Meropenem intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.125 Endotoxin Unit per mg of meropenem.

Meropenem intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store in airtight containers, at a temperature not exceeding 25°.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Meropenem Injection

Meropenem Injection is a sterile material consisting of Meropenem and Sodium Carbonate.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Meropenem Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of meropenem, $C_{17}H_{25}N_3O_5S$.

Usual strengths. 125 mg; 250 mg; 500 mg; 1 g.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 7.3 to 8.3, determined in 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 1.0 ml of *triethylamine* in 900 ml of *water*. Adjusted to pH 5.0 with *dilute phosphoric acid* and dilute to 1000 ml with *water*.

NOTE — Prepare the solutions immediately before use.

Test solution. Determine the weight of the contents of 10 containers. Dissolve a weighed quantity of the mixed contents of the 10 containers containing about 50 mg of Meropenem in 10 ml of the solvent mixture, and mix.

Reference solution. A 0.0025 per cent w/v solution of *meropenem IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature 40°,
- mobile phase: mix 1.0 ml of *triethylamine* and 900 ml of *water*, adjusted to pH 5.0 with *dilute phosphoric acid*, dilute to 1000 ml with *water*; filter and mix with 60 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the column efficiency is not less than 2500 theoretical plates.

Inject the reference solution and the test solution. Any individual impurity is not more than 0.8 per cent and the sum of all impurities found is not more than 2.0 per cent.

Content of Sodium. 80 to 120 per cent of the labelled amount of sodium.

Weigh a quantity of the injection containing 50 mg of anhydrous meropenem and dissolve in sufficient *water* to produce 100.0 ml. Dilute the resulting solution appropriately with *water* and determine by Method A for flame photometry (2.4.4), measuring at 589 nm or by Method A for atomic absorption spectrophotometry (2.4.2), using *sodium solution FP*, suitably diluted with *water* for the reference solutions.

Bacterial endotoxins (2.2.3). Not more than 0.125 Endotoxin Unit per mg of meropenem.

Sterility (2.2.11). Complies with the test for sterility.

Loss on drying (2.4.19). 9.0 per cent to 12.0 per cent, determined on 1.0 g by drying it in vacuum oven at 65° for 6 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Determine the weight of the contents of 10 containers. Dissolve a weighed quantity of the mixed contents of the 10 containers containing about 10 mg of

Meropenem in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *meropenem IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 153 volumes of buffer solution prepared by dissolving 20 ml of 25 per cent w/v of *tetrabutylammonium hydroxide* to 1000 ml with *water*, adjusted to pH 7.5 with *dilute phosphoric acid*, 30 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5, the column efficiency in not less than 2500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

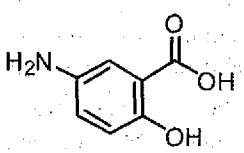
Calculate the content of $C_{17}H_{25}N_3O_5S$ in the injection.

Storage. Store protected from moisture.

Labelling. The label states the quantity in mg, of meropenem and sodium in a suitable dose-volume.

Mesalazine

Mesalamine



$C_7H_7NO_3$ Mol. Wt. 153.1

Mesalazine is 5-amino-2-hydroxybenzoic acid.

Mesalazine contains not less than 98.5 per cent and not more than 101.5 per cent of $C_7H_7NO_3$, calculated on the dried basis.

Category. Antiulcer.

Description. An almost white or light grey or light pink powder or crystals.

Identification

Tests A and C may be omitted if test B is carried out. Test B may be omitted if tests A and C are carried out.

A. Dissolve 50 mg in 10 ml of a 1.03 per cent w/v solution of *hydrochloric acid* and dilute to 100.0 ml with the same acid. Dilute 5.0 ml to 200.0 ml with a 1.03 per cent w/v solution of *hydrochloric acid*. When examined in the range 210 nm to 250 nm (2.4.7), the solution shows an absorption maximum at about 230 nm. The specific absorbance at the maximum is 430 to 450.

B. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mesalazine IPRS* or with the reference spectrum of mesalazine.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *glacial acetic acid*, 40 volumes of *methanol* and 50 volumes of *methyl isobutyl ketone*.

Solvent mixture. Equal volumes of *glacial acetic acid* and *water*.

Test solution. Dissolve 50 mg of the substance under examination in 10 ml of the solvent mixture and dilute to 20.0 ml with *methanol*.

Reference solution. Dissolve 50 mg of *mesalazine IPRS* in 10 ml of the solvent mixture and dilute to 20.0 ml with *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Light absorption. *Maintain the solutions at 40° during preparation and measurements.*

Dissolve 0.5 g in *1 M hydrochloric acid* and dilute to 20 ml with the same acid. The solution is clear (2.4.1). Immediately measure the absorbance (2.4.7) of the solution at 440 nm and 650 nm. The absorbance is not more than 0.15 at 440 nm and 0.10 at 650 nm.

Reducing substances. Dissolve 0.1 g in *dilute hydrochloric acid* and dilute to 25 ml with the same acid. Add 0.2 ml of *starch solution* and 0.25 ml of *0.01 M iodine*. Allow to stand for 2 minutes. The solution is blue or violet-brown.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions and mobile phases.

Test solution. Dissolve 50 mg of the substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b). A 0.005 per cent w/v solution of *3-aminobenzoic acid* in mobile phase A. Dilute 1.0 ml to 25.0 ml with the test solution.

Reference solution (c). A 0.0001 per cent w/v solution of *3-aminobenzoic acid* in mobile phase A.

Reference solution (d). A 0.0002 per cent w/v solution of *3-aminophenol* in mobile phase A.

Reference solution (e). A 0.0001 per cent w/v solution of *2,5-dihydroxybenzoic acid* in mobile phase A.

Reference solution (f). A 0.0003 per cent w/v solution of *salicylic acid* in mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 2.2 g of *perchloric acid* and 1.0 g of *orthophosphoric acid* in *water* and dilute to 1000.0 ml with the same solvent,

  B. dissolve 1.7 g of *perchloric acid* and 1.0 g of *orthophosphoric acid* in *acetonitrile* and dilute to 1000.0 ml with the same solvent,
- a gradient programme using the conditions given below,
- flow rate: 1.25 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
7	100	0
25	40	60
30	100	0
40	100	0

Name	Relative retention time
Mesalazine impurity B[1]	0.8
Mesalazine (Retention time: about 5 minutes)	1.0
Mesalazine impurity D[2]	1.2
Mesalazine impurity G[3]	3.1
Mesalazine impurity H[4]	3.9

[1] 3-aminophenol,

[2] 3-aminobenzoic acid,

[3] 2,5-dihydroxybenzoic acid,

[4] salicylic acid.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 1.5, where H_p is the height above the baseline of the peak due to impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

Inject reference solution (a), (c), (d), (f) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to mesalazine impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The area of any peak corresponding to mesalazine impurity D is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The area of any peak corresponding to mesalazine impurity G is not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.1 per cent). The area of any peak corresponding to mesalazine impurity H not more than the area of the principal peak in the chromatogram obtained with reference solution (f) (0.3 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Impurities A and C. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared mobile phases.

Test solution. Dissolve 50 mg of the substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a). Dissolve 5 mg of *2-aminophenol* in mobile phase A and dilute to 100.0 ml with mobile phase A. Dilute 10.0 ml to 100.0 ml with mobile phase A.

Reference solution (b). Dissolve 5 mg of *4-aminophenol* in mobile phase A and dilute to 250.0 ml with mobile phase A. To 1.0 ml of the solution, add 1.0 ml of reference solution (a) and dilute to 100.0 ml with mobile phase A.

Reference solution (c). Dilute 1.0 ml of the test solution to 200.0 ml with mobile phase A. To 5.0 ml of the solution add 5.0 ml of reference solution (a).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. dissolve 2.2 g of *perchloric acid* and 1.0 g of *orthophosphoric acid* in *water* and dilute to 1000.0 ml with *water*,

  B. dissolve 1.7 g of *perchloric acid* and 1.0 g of *orthophosphoric acid* in *acetonitrile* and dilute to 1000.0 ml with the same solvent,

– a gradient programme using the conditions given below,
– flow rate: 1 ml per minute,
– spectrophotometer set at 220 nm,
– injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
8	100	0
25	40	60
30	100	0
40	100	0

Name	Relative retention time
Mesalazine impurity A[1]	0.5
Mesalazine impurity C[2]	0.9
Mesalazine (Retention time: about 9 minutes)	1.0

[1]4-aminophenol,

[2]2-aminophenol.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to mesalazine impurity C and mesalazine is not less than 3.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak corresponding to mesalazine impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (200 ppm) and the area of any secondary peak corresponding to mesalazine impurity C is not more than 4 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (200 ppm).

Impurity K. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution. Dissolve 27.8 mg of *aniline hydrochloride* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 0.2 ml of the solution to 20.0 ml with the mobile phase. Further dilute 0.2 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system
– a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
– column temperature: 40°,
– mobile phase: a mixture of 15 volumes of *methanol* and 85 volumes of a solution containing 0.141 per cent w/v of *potassium dihydrogen phosphate* and 0.047 per cent w/v of *disodium hydrogen phosphate dihydrate* previously adjusted to pH 8.0 with a 4.2 per cent solution of *sodium hydroxide*,
– flow rate: 1 ml per minute,
– spectrophotometer set at 205 nm,
– injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the signal-to-noise ratio is not less than 10 for the principal peak.

Inject the reference solution and the test solution. The retention time of mesalazine impurity K (aniline) peak is about 15 minutes. In the chromatogram obtained with the test solution, the area of any peak corresponding to mesalazine impurity K is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (10 ppm).

Chlorides. Not more than 0.1 per cent.

Dissolve 1.5 g in 50 ml of *anhydrous formic acid.* Add 100 ml of *water* and 5 ml of *2 M nitric acid.* Titrate with *0.005 M silver nitrate* determining the end-point potentiometrically (2.4.25).

1 ml of *0.005 M silver nitrate* is equivalent to 0.1773 mg of Cl.

Sulphates (2.3.17). Disperse 1.0 g with 20 ml of *water* for 1 minute and filter. 15 ml of the filtrate complies with the limit test for sulphates (200 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 50 mg in 100 ml of boiling *water.* Cool rapidly to room temperature and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01531 g of $C_7H_7NO_3$.

Storage. Store protected from light and moisture.

Mesalazine Prolonged-release Tablets

Mesalamine Prolonged-release Tablets

Mesalazine Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Mesalazine Prolonged-release Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mesalazine, $C_7H_7NO_3$.

Usual strengths. 500 mg; 1000 mg; 1200 mg.

Identification

Boil a quantity of the powdered tablets containing 1.0 g of Mesalazine with 50 ml of *water* for 1 minute and filter the hot supernatant fluid. Cool the solution to room temperature, allow to stand, filter and dry the crystals at 110°. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mesalazine IPRS* or with the reference spectrum of mesalazine.

Tests

Dissolution (2.5.2). Complies the test stated under tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Mesalazine in 30 ml of *0.01 M hydrochloric acid* with the aid of ultrasound for 10 minutes and dilute to 50.0 ml with *0.01 M hydrochloric acid*, filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *0.01 M hydrochloric acid*. Dilute 1.0 ml of the solution to 10.0 ml with *0.01 M hydrochloric acid*.

Reference solution (b). Dilute 1.0 ml of a 0.01 per cent w/v solution of *3-aminosalicylic acid* in *0.01 M hydrochloric acid* to 100.0 ml with the test solution.

Reference solution (c). Dilute 3.0 ml of reference solution (a) to 10.0 ml with *0.01 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a 0.69 per cent w/v solution of *sodium dihydrogen phosphate monohydrate*, adjusted to pH 6.2 with *sodium hydroxide*,
  B. a mixture of 40 volumes of *acetonitrile* and 60 volumes of mobile phase A,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
8	100	0
20	85	15
40	25	75
60	0	100
61	100	0
70	100	0

Name	Relative retention time	Correction factor
Mesalazine impurity O[1]	0.55	0.6
Mesalazine impurity J[2]	0.6	2.0
Mesalazine impurity E[3]	0.8	1.3
Mesalazine (Retention time: about 6 minutes)	1.0	—
Mesalazine impurity F[4]	1.36	—
Mesalazine impurity G[5]	1.4	1.4
Mesalazine impurity P[6]	1.5	0.6
Mesalazine impurity L[7]	2.0	4.5
Mesalazine impurity M[8]	3.3	1.7
Mesalazine impurity H[9]	3.5	1.4
Mesalazine impurity R[10]	5.1	1.3
Mesalazine impurity N[11]	5.5	—

[1]unknown,

[2]diaminosalicylic acid,

[3]4-aminosalicylic acid,

[4]3-aminosalicylic acid,

[5]2,5-dihydroxybenzoic acid,

[6]unknown,

[7]2-chlorobenzoic acid,

[8]2-chloro-5-nitrobenzoic acid,

[9]salicylic acid,

[10]unknown,

[11]5-nitrosalicylic acid.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 3.0, where H_p is the height above the baseline of the peak due to 3-aminosalicylic acid and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

Inject reference solution (a), (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to mesalazine impurity H is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The area of any peak corresponding to mesalazine impurities E, F, G, J, L, M, P is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent).

Impurities A and C. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared mobile phases.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of Mesalazine in 30 ml of mobile phase A with the aid of ultrasound for 10 minutes and dilute to 50.0 ml with mobile phase A, filter.

Reference solution (a). Dilute 1.0 ml of a 0.002 per cent w/v solution of *4-aminophenol* in mobile phase A with 1.0 ml of 0.002 per cent w/v of *2-aminophenol* in mobile phase A and dilute to 100.0 ml with mobile phase A.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with mobile phase A. To 1.0 ml of the solution, add 1.0 ml of 0.0005 per cent w/v of *2-aminophenol* in mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a 0.22 per cent w/v of *perchloric acid* and 0.1 per cent w/v of *orthophosphoric acid*,
  B. a 0.17 per cent w/v of *perchloric acid* and 0.1 per cent w/v of *orthophosphoric acid* in *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
8	100	0
25	40	60
30	100	0
40	100	0

Name	Relative retention time
Mesalazine impurity A[1]	0.5
Mesalazine impurity C[2]	0.9
Mesalazine (Retention time: about 9 minutes)	1.0

[1]4-aminophenol,

[2]2-aminophenol.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to 4-aminophenol is not more than the area of corresponding peak in the chromatogram obtained with reference solution (a) (200 ppm) and the area of any peak corresponding to 2-aminophenol is not more than the area of corresponding peak in the chromatogram obtained with reference solution (a) (200 ppm).

Impurity K. Determine by liquid chromatography (2.4.14).

Test solution. Add 2 ml of *0.01 M sodium hydroxide* and 5 drops of *1 M sodium hydroxide* to a quantity of the powdered tablets containing 50 mg of Mesalazine, add 15 ml of the mobile phase and mix for 20 minutes with the aid of ultrasound and dilute to 25.0 ml with the mobile phase.

Reference solution. A 0.00000278 per cent w/v solution of *aniline hydrochloride* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 15 volumes of *methanol* and 85 volumes of a solution containing 0.141 per cent w/v of *potassium dihydrogen orthophosphate* and 0.047 per cent w/v of *disodium hydrogen orthophosphate dihydrate*, previously adjusted to pH 8.0 with 4.2 per cent w/v of *sodium hydroxide*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 50 µl.

The retention time of aniline is about 15 minutes.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to aniline is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (10 ppm).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of Mesalazine in 15 ml of *0.1 M hydrochloric acid* with the aid of ultrasound for 50 minutes with additional vortex mixing at 10 minute intervals and dilute to 25.0 ml with *0.1 M hydrochloric acid*, filter. Dilute 1.0 ml of the solution to 50.0 ml with *0.1 M hydrochloric acid.*

Reference solution (a). A 0.002 per cent w/v solution of *mesalazine IPRS* in *0.1 M hydrochloric acid.*

Reference solution (b). A 0.01 per cent w/v solution of *3-aminosalicylic acid* in *0.1 M hydrochloric acid.* Dilute

1.0 ml of the solution to 100.0 ml with 0.1 per cent w/v solution of *mesalazine IPRS* in *0.01 M hydrochloric acid.*

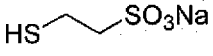
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a 0.69 per cent w/v solution of *sodium dihydrogen phosphate monohydrate,* adjusted to pH 6.2 with *dilute sodium hydroxide solution*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 3.0, where H_p is the height above the baseline of the peak due to 3-aminosalicylic acid and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to mesalazine.

Inject reference solution (a) and the test solution.

Calculate the content of $C_7H_7NO_3$ in the tablets.

Mesna



$C_2H_5NaO_3S_2$ Mol Wt.164.2

Mesna is sodium 2-sulfanylethanesulfonate.

Mesna contains not less than 96.0 per cent and not more than 102.0 per cent of $C_2H_5NaO_3S_2$, calculated on the dried basis.

Category. It works as a chemotherapeutic adjuvant. It helps in detoxifying the metabolic products of cyclophosphamide.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare th.e spectrum with that obtained with *mesna IPRS* or with reference spectrum of mesna.

B. It gives the reactions of sodium salts (2.3.1).

Tests

Solution A. A 20.0 per cent w/v solution in *carbon dioxide-free water.*

Appearance of solution. Solution A is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 4.5 to 6.0, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). A 0.0008 per cent w/v solution of *mesna impurity C IPRS [2-(acetylsulfanyl)ethanesulphonic acid]* in *the mobile phase.*

Reference solution (b). A 0.012 per cent w/v solution of *mesna impurity D IPRS (2,2'-(disulfanediyl)bis(ethanesulphonic acid)* in *the mobile phase.*

Reference solution (c). Dilute the test solution in mobile phase to obtain a solution containing 0.12 per cent w/v of mesna.

Reference solution (d). Dilute reference solution (c) in the mobile phase to obtain a solution containing 0.0012 per cent w/v of mesna.

Reference solution (e). Dilute 6.0 ml of reference solution (c) to 20.0 ml with the mobile phase. To 10 ml of the solution add 10 ml of reference solution (a).

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a buffer solution prepared by dissolving 2.94 g of *potassium dihydrogen phosphate*, 2.94 g of *dipotassium hydrogen phosphate* and 2.6 g of *tetrabutylammonium hydrogen sulphate* in about 600.0 ml of *water*, adjusted to pH 2.3 with *orthophosphoric acid*, add 335 ml of *methanol* and dilute to 1000 ml with *water.*
- flow rate: 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

The relative retention time with reference to mesna (retention time about 4.8 minutes) for *mesna impurity A IPRS (2-(carbamimidoylsulfanyl) ethanesulphonic acid)* and *mesna impurity B IPRS (2-[[(guanidino)(imino)methyl]sulfanyl] ethanesulphonic acid) are about 0.6, for mesna impurity E IPRS (2-(4,6-diamino-1,3,5-triazin-2-yl)sulfanylethane sulphonic acid)* is about 0.8, for *mesna impurity C IPRS (2-(acetylsulfanyl) ethanesulfonic acid)* is about 1.4 and for *mesna impurity D IPRS (2,2'-(disulfanediyl)bis (ethanesulfonic acid)*, is about 2.3.

Inject reference solution (e). The test is not valid unless the resolution between the peaks due to mesna and mesna impurity C is not less than 3.0.

Inject reference solution (a),(b),(d) and the test solution. In the chromatogram obtained with the test solution, the area of

the peak due to mesna impurity C is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of the peak due to mesna impurity D is not more than the area of the peak in the chromatogram obtained with reference solution (b) (3.0 per cent),the area of the peak due to mesna impurity A, B and E multiplied with correction factor 0.01 is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (d) (0.3 per cent), the area of any other secondary peak is not more than 0.33 times the area of the peak obtained with reference solution (d) (0.1 per cent) and the sum of areas all the secondary peaks is not more than the area of the peak obtained with reference solution (d) (0.3 per cent). Ignore any peak with an area less than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.045 per cent).

Chlorides (2.3.12). Dilute 5.0 ml of solution A to 15 ml with *water*. The solution complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). Dilute 5.0 ml of solution A to 30 ml with *water*. 15 ml of the solution complies with the limit for sulphates (300 ppm).

Disodium edetate. Not more than 500 ppm.

Dissolve 4.0 g in 90 ml of *water* and adjusted to pH 4.5 using *0.1 M hydrochloric acid*. Add 10 ml of *acetate buffer solution pH 4.5* and 50 ml of *2-propanol*. Add 2 ml of 0.025 per cent w/v solution of *dithizone* in *2-propanol*. Titrate with *0.01 M zinc sulphate* until the colour changes from bluish-grey to pink.

1 ml of *0.01 M zinc sulphate* is equivalent to 0.00372 g of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

Heavy metals (2.3.13). Not more than 10 ppm.

Dilute 10 ml of solution A to 20.0 ml with *water*. 12 ml of the solution complies with limit test for heavy metals, Method D (10 ppm). Use 10 ml of *lead standard solution (1 ppm Pb)* to prepare the standard.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in vacuum oven at 60° for 2 hours.

Assay. Dissolve 0.120 g in 10 ml of *water*. Add 10 ml of *1 M sulphuric acid* and 20.0 ml of *0.05 M iodine*. Titrate with *0.1 M sodium thiosulphate*, using 1 ml of *starch solution*, added towards the end of the titration as an indicator. Carry out a blank titration.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.01642 g of $C_2H_5NaO_3S_2$.

Storage. Store in tight containers.

Mesna Tablets

Mesna Tablets contain not less than 90.0 per cent and not more than 105.0 per cent of the stated amount of mesna, $C_2H_5NaO_3S_2$.

Usual strengths. 400 mg and 600 mg.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mesna IPRS* or with reference spectrum of mesna.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.06 M hydrochloric acid,*
Speed and time. 50 rpm for 15 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution (a). A 0.08 per cent w/v solution of *mesna IPRS* in the dissolution medium.

Reference solution (b). A solution containing 0.4 per cent w/v of *mesna IPRS* and 0.002 per cent w/v of *mesna related compound A IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 2.1 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 70 volumes of a buffer solution prepared by dissolving 2.72 g of *monobasic potassium phosphate* and 6.79 g of *tetrabutyl ammonium hydrogen sulphate* in 700 ml of *water* and 30 volumes of *methanol*, adjusted to pH 2.8,
- flow rate: 0.325 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 5 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between mesna and mesna related compound A is not less than 1.5 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_2H_5NaO_3S_2$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_2H_5NaO_3S_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.4 g of Mesna in 70 ml of the mobile phase, with the aid of ultrasound for 20 minutes and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.002 per cent w/v of *mesna IPRS* and 0.01 per cent w/v of *mesna related compound B IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.4 per cent w/v of *mesna IPRS* and 0.002 per cent w/v of *mesna related compound A IPRS* in the mobile phase.

Use chromatographic system as described under Dissolution with the following modification.

- spectrophotometer set at 230 nm (for reference solution (b)),
- spectrophotometer set at 202 nm (for reference solution (a) and test solution),

Name	Relative retention time
Thiouronium ethanesulphonic acid[1,2]	0.6
Guanidinethiouronium ethanesulphonic acid[1,3]	0.6
Mesna	1.0
Mesna related compound A[4]	1.3
Mesna related compound B[5]	2.5

[1]Process related impurity not included in total impurities;

[2]2-(Carbamimidoylthio)ethane-1-sulphonic acid;

[3]2-[(*N*-Carbamimidoylcarbamimidoyl)thio]ethane-1-sulphonic acid;

[4]2-(Acetylthio)ethane-1-sulphonic acid;

[5]2,2-Disulfanediylbis(ethane-1-sulphonic acid).

Inject reference solution (a) and (b). The test is not valid unless the resolution between the mesna and mesna related compound A is not less than 1.5 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections for mesna and mesna related compound B is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to mesna related compound B is not more than 1.2 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (3.0 per cent), the area of any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks other than mesna related compound B is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.4 g of Mesna in 70 ml of the mobile phase, with the aid of ultrasound for 20 minutes and dilute to 100.0 ml with the mobile phase.

Reference solution (a). A 0.4 per cent w/v solution of *mesna IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.4 per cent w/v of *mesna IPRS* and 0.002 per cent w/v of *mesna related compound A IPRS* in the mobile phase.

Use chromatographic system as described under Dissolution.

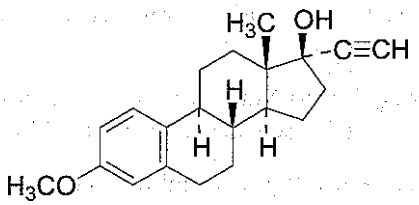
Inject reference solution (a) and (b). The test is not valid unless the resolution between the mesna and mesna related compound A is not less than 1.5 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_2H_5NaO_3S_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Mestranol



$C_{21}H_{26}O_2$ Mol. Wt. 310.4

Mestranol is 3-methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17β–ol.

Mestranol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{26}O_2$, calculated on the dried basis.

Category. Estrogen.

Description. A white or almost white, crystalline powder.

Identification

Test B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mestranol IPRS* or with the reference spectrum of mestranol.

B. In the test for Related substances the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

C. Dissolve about 5 mg in 1 ml of *sulphuric acid*; a red colour is produced which appears greenish-yellow under ultraviolet light at 365 nm. On adding the solution to 10 ml of *water* and mixing, the solution becomes pink and on standing a pink to violet precipitate is produced.

Tests

Specific optical rotation (2.4.22). –24.0° to –20.0°, determined in a 1.0 per cent w/v solution in *anhydrous pyridine*.

Light absorption (2.4.7). Dissolve about 25 mg in sufficient *ethanol (95 per cent)* to produce 25 ml and dilute 10 ml of the solution to 100 ml with *ethanol (95 per cent)*. When examined in the range 230 nm to 360 nm, the resulting solution shows absorption maxima at about 279 nm and 288 nm and a minimum at about 286 nm. Absorbance at about 279 nm is 0.062 to 0.068 and at about 288 nm is 0.059 to 0.064, both calculated on the dried basis.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (95 per cent)*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml of *chloroform*.

Test solution (b). Dissolve 0.1 g of the substance under examination in 100 ml of *chloroform*.

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution (b). A 0.005 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution (c). A 0.1 per cent w/v solution of *mestranol IPRS* in *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, heat it at 110° for 10 minutes and spray the hot plate with *ethanolic sulphuric acid* (20 per cent). Heat again at 110° for 10 minutes and examine in daylight and under ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a), and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

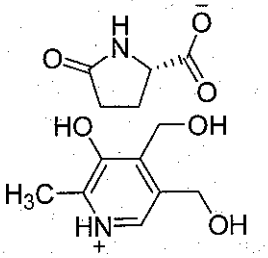
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

Assay. Weigh 0.2 g, dissolve in 40 ml of *tetrahydrofuran* and add 5 ml of 10 per cent w/v solution of *silver nitrate*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03104 g of $C_{21}H_{25}O_2$.

Storage. Store protected from light and moisture.

Metadoxine



$C_{13}H_{18}N_2O_6$ Mol. Wt. 298.3

Metadoxine is 5-oxo-L-proline- 4, 5-bis (hydroxymethyl)-2-methylpyridin-3-ol.

Metadoxine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{13}H_{18}N_2O_6$, calculated on the dried basis.

Category. Hepatoprotective.

Description. A white to off white, powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metadoxine IPRS* or with the reference spectrum of metadoxine.

Tests

pH (2.4.24).3.8 to 4.2, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). – 8.5° to – 6.5°, determined in a 5.0 per cent w/v solution in *0.1 M hydrochloric acid*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination and dilute to 50.0 ml with the *water*.

Reference solution. A 0.01 per cent w/v solution of *metadoxine IPRS* in *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) (Such as Hypersil BDS),
- mobile phase: Dissolve 8.6 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusted to pH 7.0 with *potassium hydroxide solution*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

The retention time of Metadoxine is about 15 minutes.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 3.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. The area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram with the reference solution (1.0 per cent). Ignore the peak due to pyroglutamic acid at the relative retention time of about 0.24.

Chlorides (2.3.12). Dissolve 0.25 g in *water* and dilute to 25 ml with *water*. The solution complies with the limit test for chlorides (1000 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.3 per cent.

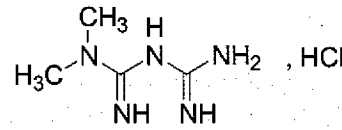
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in oven at 60° for 5 hours.

Assay. Weigh accurately about 0.24 g and dissolve in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of 0.1 M perchloric acid is equivalent to 0.02983 g of $C_{13}H_{18}N_2O_6$.

Storage. Store protected from light and moisture.

Metformin Hydrochloride



$C_4H_{11}N_5,HCl$ Mol. Wt. 165.6

Metformin Hydrochloride is 1,1-dimethylbiguanide hydrochloride.

Metformin Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_4H_{11}N_5,HCl$, calculated on the dried basis.

Category. Hypoglycaemic.

Description. A white or almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metformin hydrochloride IPRS* or with the reference spectrum of metformin hydrochloride.

B. Dissolve 25 mg in 5 ml of *water*, add 1.5 ml of *5 M sodium hydroxide*, 1 ml of *1-naphthol solution* and, dropwise with shaking, 0.5 ml of *sodium hypochlorite solution (3 per cent Cl)*; an orange-red colour is produced which darkens on keeping.

C. Dissolve 10 mg in 10 ml of *water* and add 10 ml of a solution prepared by mixing equal volumes of a 10 per cent w/v solution of *sodium nitroprusside*, a 10 per cent w/v solution of *potassium ferricyanide* and a 10 per cent w/v solution of *sodium hydroxide* and allowing to stand for 20 minutes; a wine red colour develops within 3 minutes.

D. It gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 10 ml of *water*.

Reference solution (a). A 0.0005 per cent w/v solution of the substance under examination in *water*.

Reference solution (b). A 0.0001 per cent w/v solution of *dicyandiamide* in *water*.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),

- mobile phase: a solution containing 0.087 per cent w/v of *sodium pentanesulphonate* and 0.12 per cent w/v of *sodium chloride*, adjusted to pH 3.5 using 1 per cent v/v solution of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume: 20 µl.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to dicyandiamide is not more than the area of the peak in the chromatogram obtained with reference solution (b) (0.02 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay Dissolve 0.1 g in 4 ml of *anhydrous formic acid*. Add 80 ml of *acetonitrile* and titrate with *0.1M perchloric acid* immediately. Determine the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01656 g of $C_4H_{12}ClN_5$.

Storage. Store protected from light and moisture.

Metformin Oral Solution

Metformin Hydrochloride Oral Solution

Metformin Oral Solution is a solution of Metformin Hydrochloride in a suitable flavoured vehicle.

Metformin Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metformin hydrochloride, $C_4H_{11}N_5,HCl$.

Usual strength. 100 mg per 5ml.

Identification

In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 6.0 to 7.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of oral solution with the mobile phase to obtain a solution containing 0.5 per cent w/v of Metformin Hydrochloride and filter, if necessary.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 20 mg of *dicyandiamide* in *water*, dilute to 100 ml with *water*. Dilute 1.0 ml of the solution to 200.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.00025 per cent w/v of *melamine* and 0.0001 per cent w/v of *metformin hydrochloride IPRS* in the mobile phase.

Reference solution (d). Dilute 1 volume of reference solution (a) to 2 volumes with the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with benzenesulphonic acid groups bonded to porous silica (5 µm) (Such as Partisphere SCX),
- mobile phase: a 1.7 per cent w/v solution of *ammonium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume: 20 µl.

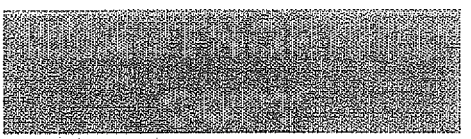
Inject reference solution (c). The test is not valid unless the resolution between the peaks due to melamine and metformin hydrochloride is not less than 10.0.

Inject reference solution (a), (b), (d) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to dicyandiamide is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent), the area of any other secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent) and the sum of the areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent). Ignore any peak, except that of dicyandiamide, with an area less than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Other tests. Comply with the tests stated under Oral liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of oral solution with the mobile phase to obtain a solution containing 0.05 per cent w/v of Metformin Hydrochloride and filter, if necessary.



Reference solution (a). A 0.05 per cent w/v solution of *metformin hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.00025 per cent w/v of *melamine* and 0.0001 per cent w/v of *metformin hydrochloride IPRS* in the mobile phase.

Use chromatographic system as described in the Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to melamine and metformin hydrochloride is not less than 10.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_4H_{11}N_5,HCl$, weight in volume.

Metformin Hydrochloride Prolonged-release Tablets

Metformin Hydrochloride Sustained-release Tablets; Metformin Hydrochloride Extended-release Tablets

Metformin Hydrochloride Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Metformin Hydrochloride Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metformin hydrochloride, $C_4H_{11}N_5,HCl$.

Usual strengths. 100 mg; 500 mg; 850 mg; 1 g.

Identification

A. Shake a quantity of the powdered tablets containing about 20 mg of Metformin Hydrochloride with 20 ml of *dehydrated alcohol* and filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 105° for 1 hour. The residue complies with the following test. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metformin hydrochloride IPRS* or with the reference spectrum of metformin hydrochloride.

B. Triturate a quantity of the powdered tablets containing about 50 mg of Metformin Hydrochloride with 10 ml of *water* and filter. To 5 ml of the filtrate, add 1.5 ml of *5 M sodium hydroxide*, 1 ml of *1-naphthol solution* and, dropwise with shaking, 0.5 ml of *dilute sodium hypochlorite solution;* an orange-red colour is produced which darkens on keeping.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 2.5 per cent v/v solution of *acetonitrile* in *water*.

Test solution. Disperse a quantity of the powdered tablets containing about 0.5 g of Metformin Hydrochloride in 100 ml of the solvent mixture. Take 10 ml of the solution and centrifuge at 3500 rpm for 15 minutes. Dilute 5 ml of this supernatant to 100 ml with the solvent mixture.

Reference solution (a). A 0.0005 per cent w/v solution of *dicyandiamide* in the solvent mixture.

Reference solution (b). A 0.0025 per cent w/v solution of *metformin hydrochloride IPRS* in the solvent mixture.

Reference solution (c). Dilute 1 ml each of reference solution (a) and reference solution (b), to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of buffer solution prepared by dissolving 1 g each of *sodium heptane sulphonate* and *sodium chloride* to 1800 ml of *water,* adjusted to pH 3.85 with *0.06 M orthophosphoric acid* and dilute to 2000 ml with *water* and 10 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume: 20 µl.

Inject reference solution (c). The test is not valid unless the tailing factor of the peak due to metformin hydrochloride is not more than 2.0. The resolution between the peaks due to dicyandiamide and metformin hydrochloride is not less than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent for metformin hydrochloride and not more than 10 per cent for dicyandiamide.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution the area of peak corresponding to dicyandiamide is not more than the area of the peak due to dicyandiamide in the chromatogram obtained with reference solution (c) (0.02 per cent) and the area of any other secondary peak is not more than the area of peak due to metformin hydrochloride in the chromatogarm obtained with reference solution (c) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.1 g of Metformin Hydrochloride with 70 ml of *water* until complete dispersion, dilute to 100.0 ml with *water* and filter. Dilute 5 ml of the filtrate to 50.0 ml with *water*. Further dilute 5.0 ml to 50.0 ml with *water*. Measure the

absorbance of the resulting solution at the maximum at about 232 nm (2.4.7).

Calculate the content of $C_4H_{11}N_5HCl$ from the absorbance obtained by carrying out the Assay simultaneously using *metformin hydrochloride IPRS*.

Metformin Hydrochloride Prolonged-release and Glimepiride Tablets

Metformin Hydrochloride Prolonged-release and Glimepiride Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Metformin Hydrochloride prolonged-release and Glimepiride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of metformin hydrochloride, $C_4H_{11}N_5,HCl$ and glimepiride, $C_{24}H_{34}N_4O_5S$.

Usual strengths. Glimepiride, 1.0 mg and Metformin Hydrochloride, 500 mg; Glimepiride, 2.0 mg and Metformin Hydrochloride, 500 mg.

Identification

A. When examined in the range of 200 nm to 300 nm, a 0.001 per cent w/v solution of *metformin hydrochloride IPRS* and test solution, as obtained in the Assay, shows absorption maxima, at about 232 nm (2.4.7).

B. In the Assay of Glimepiride, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

For Metformin hydrochloride — Complies with the test stated under Tablets.

For Glimepiride —

Apparatus No. 2 (Paddle),
Medium. 900 ml of a buffer solution prepared by dissolving 0.58 g of *monobasic potassium phosphate* and 8.86 g of anhydrous *dibasic sodium phosphate* in 1000 ml of *water* and adjusted to pH 7.8 with *orthophosphoric acid* or *1M sodium hydroxide*, add 10 g of *sodium lauryl sulphate* and mix.
Speed and time. 100 rpm and 90 minutes.

Withdraw a suitable volume of the medium and filter. Dilute further, if necessary, with the dissolution medium.

Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 90 volumes of *acetonitrile* and 10 volumes of *water*.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Weigh accurately 10 mg of *glimepiride IPRS* in 10-ml volumetric flask and dilute with solvent mixture and sonicate. Dilute further with dissolution medium to obtain a solution containing 0.0001 per cent w/v solution of glimepiride.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 0.5 g *sodium dihydrogen phosphate* in 500 ml of *water*, adjusted to pH 2.1 to 2.7 with *orthophosphoric acid*, and 65 volumes of *acetonitrile*.
- flow rate: 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{24}H_{34}N_4O_5S$.

Q. Not less than 70 per cent of the stated amounts of $C_{24}H_{34}N_4O_5S$.

Uniformity of content. Complies with the test stated under Tablets.

For Glimepiride — Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Disperse 1 tablet in 20.0 ml of solvent mixture with the aid of ultrasound and dilute to 25.0 ml with the same solvent. Dilute further, if necessary with the solvent mixture.

Reference solution. Weigh accurately 10 mg *glimepiride IPRS* in 10-ml volumetric flask, shake to dissolve and dilute to volume with solvent mixture. Further dilute 1.0 ml of the solution to 25.0 ml with the same solvent. Dilute further, if necessary with the solvent mixture.

Inject the reference solution and test solution.

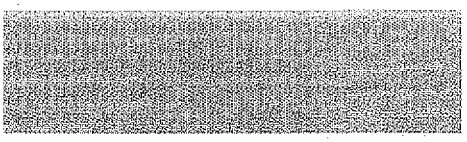
Calculate the content of $C_{24}H_{34}N_4O_5S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay

For Metformin hydrochloride —

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 50 mg Metformin Hydrochloride in 70 ml of *water* with the aid of ultrasound and dilute to 100.0 ml with *water*. Dilute 1.0 ml of the solution to 50.0 ml with *water*.



Reference solution. A 0.001 per cent w/v solution of *metformin hydrochloride IPRS* in *water*.

Measure the absorbance of the reference solution and the test solution at 232 nm (2.4.7) using *water* as blank. Calculate the content of $C_4H_{11}N_5$,HCl in tablets.

For Glimepiride — Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 90 volumes of *acetonitrile* and 10 volumes of *water*.

Test solution. Disperse intact tablets in solvent mixture with the aid of ultrasound to produce a solution containing 0.004 per cent w/v of Glimepiride.

Reference solution. Weigh accurately 10 mg *glimepiride IPRS* in 10-ml volumetric flask and dilute with solvent mixture. Transfer 1.0 ml of the solution in a 25-ml volumetric flask and dilute with solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 0.5 g *monobasic sodium phosphate* in 500 ml of *water*, adjusted to pH 2.1 to 2.7 with *orthophosphoric acid*, and 65 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume: 20 µl.

Inject the reference solution and test solution.

Calculate the content of $C_{24}H_{34}N_4O_5S$ in tablets.

Metformin Tablets

Metformin Hydrochloride Tablets

Metformin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metformin hydrochloride, $C_4H_{11}N_5$,HCl.

Usual strengths. 500 mg; 850 mg.

Identification

A. Shake a quantity of the powdered tablets containing 20 mg of Metformin Hydrochloride with 20 ml of *ethanol*, filter, evaporate the filtrate to dryness on a water-bath and dry the residue at 105° for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metformin hydrochloride IPRS* or with the reference spectrum of metformin hydrochloride.

B. Triturate a quantity of the powdered tablets containing 50 mg of Metformin Hydrochloride with 10 ml of *water* and filter. To 5 ml of the filtrate, add 1.5 ml of *5 M sodium hydroxide*, 1 ml of *1-naphthol solution* and, dropwise with shaking, 0.5 ml of *sodium hypochlorite solution (3 per cent)*; an orange-red colour is produced which darkens on keeping.

C. The filtrate obtained in test B gives reaction (A) of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),

Medium. 900 ml of a 0.68 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 6.8 by the addition of *1 M sodium hydroxide*,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter, dilute suitably with *water* and measure the absorbance of the resulting solution at the maximum at about 233 nm (2.4.7). Calculate the content of $C_4H_{11}N_5$,HCl, in the medium taking 806 as the specific absorbance at 233 nm.

Q. Not less than 70 per cent of the stated amount of $C_4H_{11}N_5$, HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 0.5 g of Metformin Hydrochloride with 100 ml of *water* and filter.

Reference solution (a). Dilute 0.1 ml of the test solution to 100 ml with *water*.

Reference solution (b). A 0.0001 per cent w/v solution of *dicyandiamide* in *water*.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a solution containing 0.087 per cent w/v of *sodium pentanesulphonate* and 0.12 per cent w/v of *sodium chloride*, adjusted to pH 3.5 using 1 per cent v/v solution of *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 218 nm,
- injection volume: 20 µl.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to dicyandiamide is not more than the area of the peak in the chromatogram obtained with reference solution (b) (0.02 per cent), the area of any other secondary peak is not more than the area of the principal peak

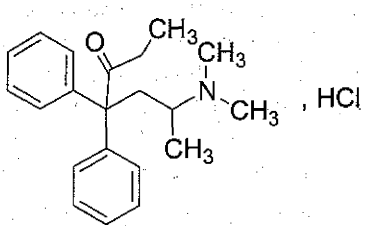
in the chromatogram obtained with reference solution (a) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.1 g of Metformin Hydrochloride, shake with 70 ml of *water* for 15 minutes, dilute to 100.0 ml with *water* and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *water*. Further dilute 10.0 ml to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 232 nm (2.4.7). Calculate the content of $C_4H_{11}N_5$, HCl taking 798 as the specific absorbance at 232 nm.

Methadone Hydrochloride

Amidone Hydrochloride



$C_{21}H_{27}NO,HCl$ Mol. Wt. 345.9

Methadone Hydrochloride is (*RS*)-dimethyl-(1-methyl-4-oxo-3,3-diphenylhexyl)amine hydrochloride.

Methadone Hydrochloride contains not less than 98.5 per cent and not more than 100.5 per cent of $C_{21}H_{27}NO,HCl$, calculated on the dried basis.

Category. Narcotic analgesic.

Description. A white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methadone hydrochloride IPRS* or with the reference spectrum of methadone hydrochloride.

B. To 2 ml of a 5 per cent w/v solution in *carbon dioxide-free water* add 1 ml of *0.1 M hydrochloric acid* and 6 ml of *ammonium thiocyanate solution*; a white precipitate is produced which becomes crystalline on stirring for a few minutes. The precipitate, after drying at 105° melts at 143° to 148° (2.4.21).

C. Dissolve 50 mg in 5 ml of *carbon dioxide-free water*, add 1 ml of *6 M ammonia*, mix, allow to stand for 5 minutes and filter; the filtrate gives reaction (A) of chlorides (2.3.1).

D. Optical rotation of a 2-dm layer of a 5 per cent w/v solution in *carbon dioxide-free water*, is −0.05° to +0.05° (2.4.22).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of a 2.0 per cent w/v solution in *carbon dioxide-free water* add 0.2 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.4 ml of *0.01 M hydrochloric acid*; the solution is red.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.1 g of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with *methanol*. Further dilute 1.0 ml of the solution to 100.0 ml with *methanol*.

Reference solution (b). A solution containing 0.005 per cent w/v each of *imipramine hydrochloride IPRS* and *cyclobenzaprine hydrochloride IPRS* in *methanol*.

Chromatographic system

- a fused silica column 50 m x 0.32 mm, packed with poly(dimethyl)(diphenyl)siloxane (film thickness 1.05 µm),
- temperature:

column	time (min.)	temperature (°)
	0-4	150→250
	4-35	250

- inlet port at 200° and detector at 250°,
- split ratio. 1:100,
- flame ionization detector,
- flow rate: 1.2 ml per minute, using nitrogen as the carrier gas.

Name	Relative retention time
Methadone impurity E[1]	0.44
Methadone impurity C[2]	0.81
Methadone impurity B[3]	0.89
Methadone impurity D[4]	0.98
Methadone (Retention time: about 25 minutes)	1.0
Methadone impurity A[5]	1.14
Imipramine	1.19
Cyclobenzaprine	1.24

[1]diphenylacetonitrile,
[2](3*RS*)-4-(dimethylamino)-3-methyl-2,2- diphenylbutanenitrile,
[3](4*RS*)-4-(dimethylamino)-2,2- diphenylpentanenitrile,
[4]isomethadone,
[5]isomethadone ketimine.

Inject 2 µl of reference solution (b). The test is not valid unless the resolution between the peaks due to imipramine and cyclobenzaprine is not less than 3.0.

Inject 2 µl of reference solution (a) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.5 g, dissolve in 50 ml of *anhydrous glacial acetic acid*, add 5 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator and continuing the titration until the colour changes from violet-blue to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03459 g of $C_{21}H_{27}NO,HCl$.

Storage. Store protected from light and moisture.

Methadone Injection

Methadone Hydrochloride Injection; Amidone Hydrochloride Injection; Amidone Injection

Methadone Injection is a sterile solution of Methadone Hydrochloride in Water for Injections.

Methadone Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of methadone hydrochloride, $C_{21}H_{27}NO,HCl$.

Usual strengths. 5 mg per ml; 10 mg per ml.

Identification

Make a volume containing 0.1 g of Methadone Hydrochloride alkaline with *5 M sodium hydroxide*, stir with a glass rod until the precipitate solidifies, filter, wash with *water* and dry over *phosphorus pentoxide* at room temperature at a pressure of 2 kPa. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methadone hydrochloride IPRS* treated in the same manner or with the reference spectrum of methadone.

B. To 5 mg add 0.05 ml of *dinitrobenzene solution* and 0.05 ml of a 50 per cent w/v solution of *sodium hydroxide*; a purple colour is produced which changes slowly to dark brown.

Tests

pH (2.4.24). 5.0 to 6.5.

Bacterial endotoxins (2.2.3). Not more than 8.8 Endotoxin Units per mg of methadone hydrochloride.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume containing 10 mg of Methadone Hydrochloride add 1 ml of *glacial acetic acid* and dilute to 100.0 ml with *water*. To 10.0 ml of the solution add 10 ml of a 0.4 per cent w/v solution of *picric acid* and 10 ml of *phosphate buffer pH 4.9*, extract with three quantities, each of 15 ml, of *chloroform*, dilute the combined chloroform extracts to 50.0 ml with *chloroform*. To 10.0 ml add sufficient *chloroform* to produce 20.0 ml and measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7), using as the blank a solution prepared in the same manner but omitting the substance under examination. Calculate the content of $C_{21}H_{27}NO,HCl$ taking 448 as the specific absorbance at 350 nm.

Storage. Store protected from light, in single dose container.

Methadone Linctus

Methadone Hydrochloride Linctus; Amidone HydrochlorideLinctus; Amidone Linctus

Methadone Linctus is a solution containing 0.04 per cent w/v of Methadone Hydrochloride in a suitable vehicle with a tolu flavour.

Methadone Linctus contains not less than 0.036 per cent and not more than 0.044 per cent w/v of stated amount of methadone hydrochloride, $C_{21}H_{27}NO,HCl$.

Category. Opioid analgesic.

Identification

To 50 ml, add 30 ml of *water* and *1 M sulphuric acid* until the solution is acidic to *litmus paper*. Extract with two 20 ml quantities of *petroleum spirit (boiling range, 40° to 60°)*, discarding the extracts, add *5 M sodium hydroxide* until the solution is alkaline to *litmus paper*. Add 4 g of *sodium chloride*, shake to dissolve, extract with two 25 ml quantities of *ether* and wash the combined ether extracts with five 20 ml quantities of *water*. Dry with *anhydrous sodium sulphate*, filter, evaporate to dryness and dry the residue over *phosphorus pentoxide* at a pressure of 2 kPa. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methadone hydrochloride IPRS* treated in the same manner or with the reference spectrum of methadone.

B. To 5 mg add 0.05 ml of *dinitrobenzene solution* and 0.05 ml of a 50 per cent w/v solution of *sodium hydroxide*. A purple colour is produced which changes slowly to dark brown.

Tests

Other tests. Comply with the tests stated under Oral Liquids.

Assay. To 30 g add 1 ml of *glacial acetic acid* and dilute to 100 ml with *water*. To 10 ml of the resulting solution add 10 ml of a 0.4 per cent w/v solution of *picric acid* and 10 ml of *phosphate buffer pH 4.9*, extract with three 15 ml quantities of *chloroform* and dilute the combined chloroform extracts to 50 ml with *chloroform*. To 10 ml add sufficient *chloroform* to produce 20 ml and measure the absorbance of the resulting solution (2.4.7), at the maximum at about 350 nm using as the blank a solution prepared in the same manner but using 10 ml of a 1 per cent v/v solution of *glacial acetic acid* and beginning at the words 'add 10 ml of a 0.4 per cent w/v .....'. Calculate the content of $C_{21}H_{27}NO$, HCl taking 448 as the specific absorbance at 350 nm.

Determine the weight per ml (2.4.29) of the linctus, and calculate the content of $C_{21}H_{27}NO$,HCl, weight in volume.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of methadone.

Methadone Oral Solution

Methadone Hydrochloride Oral Solution; Amidone Hydrochloride Oral Solution; Amidone Oral Solution

Methadone Oral Solution contains 0.1 per cent w/v of Methadone Hydrochloride in a suitable aqueous vehicle. It is supplied as a ready-to-use solution or it is prepared from Methadone Hydrochloride Oral Concentrate in accordance with the manufacturer's instructions.

Methadone Oral Solution contains not less than 0.09 per cent w/v and not more than 0.11 per cent w/v of methadone hydrochloride, $C_{21}H_{27}NO$,HCl.

Usual strength. 0.1 per cent w/v.

Identification

In the Assay, the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Oral liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute 1 volume of the oral solution to 10 volumes with the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *methadone hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Lichrosorb RP18),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *0.02 M potassium dihydrogen orthophosphate*, adjusted to pH 5.5 with *2 M orthophosphoric acid* or *2 M sodium hydroxide*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{27}NO$,HCl in the oral solution.

Methadone Hydrochloride Oral Concentrate

Methadone Hydrochloride Oral Concentrate is a solution of Methadone Hydrochloride in a suitable aqueous vehicle.

Metformin Hydrochloride Oral Concentrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of methadone hydrochloride, $C_{21}H_{27}NO$,HCl.

Identification

In the Assay, the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Oral liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the concentrate to produce a solution containing 0.01 per cent w/v of Methadone Hydrochloride in the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *methadone hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Lichrosorb RP18),

- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *0.02 M potassium dihydrogen orthophosphate*, adjusted to pH 5.5 with *2 M orthophosphoric acid* or *2 M sodium hydroxide*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{27}NO,HCl$ in the concentrate.

Labelling. At the specific request of the prescriber, the concentrate may be diluted to a concentration other than 0.1 per cent w/v in accordance with the manufacturer's instructions.

Methadone Tablets

Methadone Hydrochloride Tablets; Amidone Hydrochloride Tablets; Amidone Tablets

Methadone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of methadone hydrochloride, $C_{21}H_{27}NO,HCl$.

Usual strengths. 5 mg; 10 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Methadone Hydrochloride with 20 ml of *water* and centrifuge. Make the supernatant liquid alkaline with *5 M sodium hydroxide*, stir with a glass rod until the precipitate solidifies, filter, wash with *water* and dry over *phosphorus pentoxide* at room temperature at a pressure of 2 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methadone hydrochloride IPRS* treated in the same manner or with the reference spectrum of methadone.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 500 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and transfer equivalent to 0.4 mg of Methadone Hydrochloride into a separating funnel. Add 1 ml of *glacial acetic acid* and 20 ml of 0.02 per cent w/v solution of *bromocresol purple* in 2 per cent v/v solution of *glacial acetic acid*. Add 20.0 ml of *chloroform*, mix and extract. Measure the absorbance of the chloroform at the maximum at 405 nm. (2.4.7). Calculate the content of $C_{21}H_{27}NO,HCl$ in the medium from the absorbance obtained from the chloroform extract similarly prepared of known concentration of *methadone hydrochloride IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{21}H_{27}NO,HCl$.

Uniformity of content. Determine by liquid chromatography (2.4.14).

Test solution. Disperse 1 intact tablet in the mobile phase, with the aid of ultrasound with intermittent shaking and dilute with the mobile phase to obtain a solution containing 0.5 per cent w/v of Methadone Hydrochloride.

Reference solution. A 0.5 per cent w/v solution of *methadone hydrochloride IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with phenyl groups bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 4.08 g of *potassium dihydrogen phosphate* in 1000 ml of *water* and 40 volumes of *acetonitrile*, adjusted to pH 3.2 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{27}NO,HCl$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Uniformity of content using the following solution.

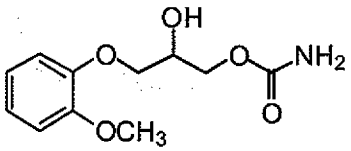
Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 10 mg of Methadone Hydrochloride in mobile phase, with the aid of ultrasound and dilute to 20.0 ml with the mobile phase.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{27}NO,HCl$ in the tablets.

Storage. Store protected from light and moisture.

Methocarbamol



$C_{11}H_{15}NO_5$ Mol. Wt. 241.2

Methocarbamol is (*RS*)-2-Hydroxy-3-(2-methoxyphenoxy) propyl carbamate.

Methocarbamol contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{11}H_{15}NO_5$, calculated on the dried basis.

Category. Muscle relaxant.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methocarbamol IPRS* or with the reference spectrum of methocarbamol.

B. When examined in the range 200 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in *methanol* shows absorption maxima at about 274 nm.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Test solution. Dissolve 100 mg of substance to under examination in 13 ml of *methanol* with the aid of ultrasound and dilute to 50.0 ml with buffer solution.

Reference solution (a). A 0.04 per cent w/v solution of *guaifenesin IPRS* in *methanol*.

Reference solution (b) .Dissolve 20.0 mg of *methocarbamol IPRS* in 2 ml of *methanol*, add 1.0 ml of reference solution (a) and dilute to 10.0 ml with buffer solution.

Reference solution (c). Dilute 1.0 ml of test solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjusted to pH 4.5 with *6 M ortho phosphoric acid* or *10 M* of *potassium hydroxide* and 25 volumes of *methanol*,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless resolution between the peaks due to guaifenesin and methocarbamol is not less than 2.0.

The relative retention time of guaifenesin with reference to methocarbamol is about 0.8.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the sum of areas of all the secondary peak is not more than the area of principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

Heavy metals (2.3.13). Dissolve 1.0 g of substance under examination in 7 ml of *methanol* and 3 ml of *1 M acetic acid*, and dilute to 25 ml with *water*. The resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° for 2 hours.

Assay. Weigh 100 mg of the substance under examination in 100 ml of *methanol* with the aid of ultrasound, filter. Dilute 4.0 ml of the solution to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 274 nm (2.4.7), using *methanol* as blank Calculate the content of $C_{11}H_{15}NO_5$ from the absorbance obtained by repeating the operation using *methocarbamol IPRS* in place of the substance under examination.

Storage. Store protected from moisture.

Methocarbamol Tablets

Methocarbamol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of methocarbamol, $C_{11}H_{15}NO_5$.

Usual strengths. 500 mg; 750 mg.

Identification

Mix a quantity of the powdered tablets containing 1g of methocarbamol with 25 ml of *water* in a separator, and extract with 25 ml of *chloroform*, filter the chloroform layer through anhydrous sodium sulphate and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methocarbamol IPRS* or with the reference spectrum of methocarbamol.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. A solution of *methocarbamol IPRS* in the dissolution medium suitably diluted to obtain a solution having the similar concentration as that of the test solution.

Use the chromatographic system as described in the Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{15}NO_5$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{11}H_{15}NO_5$.

Other tests. Complies with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Buffer solution pH 4.5. Dissolve 6.8 g of *monobasic potassium phosphate* in *water* and dilute to 1000.0 ml with *water.* Adjust to pH 4.5 with *orthophosphoric acid.*

Internal standard solution. A 0.3 per cent w/v solution of *caffeine* in *methanol.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 100 mg of methocarbamol in 50 ml of buffer solution, add 25 ml of *methanol,* 5.0 ml of internal standard solution, mix with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with the buffer solution, mix and filter.

Reference solution. Dissolve 100 mg of *methocarbamol IPRS* in 50 ml of buffer solution, add 25 ml of *methanol,* 5.0 ml of internal standard solution, mix with the aid of ultrasound for 10 minutes, dilute to 100.0 ml with buffer solution, mix.

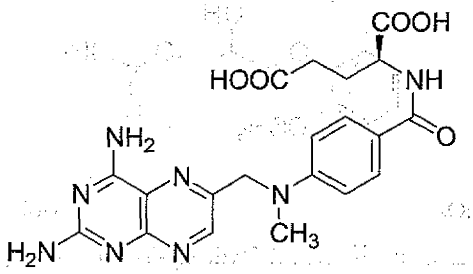
Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of buffer solution,
- flow rate: 1 ml per minute,
- spectrophotometer set at 274 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{11}H_{15}NO_5$ in the tablets.

Storage. Store protected from moisture.

Methotrexate



$C_{20}H_{22}N_8O_5$ Mol. Wt. 454.4

Methotrexate is 4-amino-4-deoxy-10-methylpteroyl-L-glutamic acid.

Methotrexate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{20}H_{22}N_8O_5$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A yellow to orange-brown, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methotrexate IPRS* or with the reference spectrum of methotrexate.

B. When examined in the range 230 nm to 380 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at about 258 nm, 303 nm and 371 nm; ratio of the absorbance at the maximum at about 303 nm to that at the maximum at about 371 nm, 2.8 to 3.3.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 0.5 ml of *dilute ammonia* and 5 ml of mobile phase A.

Test solution. Dissolve 40 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with mobile phase A.

Reference solution (a). Dilute 5.0 ml of the test solution to 100.0 ml with mobile phase A. Further dilute 5.0 ml of the solution to 50.0 ml with mobile phase A.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 25.0 ml with mobile phase A.

Reference solution (c). A solution containing 0.005 per cent w/v each of the substance under examination, *methotrexate impurity B IPRS, methotrexate impurity C IPRS, methotrexate impurity D IPRS* and *methotrexate impurity E IPRS* prepared

by dissolving in the solvent mixture and diluted with mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 5 volumes of *acetonitrile* and 95 volumes of a 0.34 per cent w/v solution of *anhydrous sodium dihydrogen phosphate* previously adjusted to pH 6.0 with a 4.2 per cent w/v solution of *sodium hydroxide*,

  B. a mixture of 50 volumes of *acetonitrile* and 50 volumes of a 0.34 per cent w/v solution of *anhydrous sodium dihydrogen phosphate* previously adjusted to pH 6.0 with a 4.2 per cent w/v solution of *sodium hydroxide*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
10	100	0
20	95	5
28	50	50
37	50	50
40	100	0

Name	Relative retention time	Correction factor
Methotrexate impurity B[1]	0.3	---
Methotrexate impurity C[2]	0.4	---
Methotrexate (Retention time: about 18 minutes)	1.0	—
Methotrexate impurity E[3]	1.4	0.8
Methotrexate impurity I[4]	1.5	1.4
Methotrexate impurity H[5]	1.6	---

[1]4-aminofolic acid,

[2]*N*-methylfolic acid,

[3]4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoic acid,

[4]methotrexate 1-methyl ester,

[5]methotrexate 5-methyl ester.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to methotrexate impurities B and C and is not less than 2.0 and between the peaks due to methotrexate impurity D and methotrexate is not less than 1.5.

Inject reference solution (a), (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to methotrexate impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of any peak corresponding to methotrexate impurities B and E is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The area of any peak corresponding to methotrexate impurities H and I is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) and the sum of areas of all the secondary peaks other than methotrexate impurities B, C and E is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.03 per cent).

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 4.0 mg of *methotrexate for system suitability IPRS* (containing impurity F) in the mobile phase and dilute to 20.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.0 mm packed with *bovine albumin* bound to *silica gel for chromatography* (7 µm) with a pore size of 30 nm
- mobile phase: add 500 ml of 0.71 per cent w/v solution of *anhydrous disodium hydrogen phosphate* to 600 ml of a 0.69 per cent w/v solution of *sodium dihydrogen phosphate monohydrate*, mix, adjusted to pH 6.9 with dilute *sodium hydroxide* solution, to 920 ml of this mixture add 80 ml of *propanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume: 20 µl.

The relative retention time with reference to methotrexate (retention time is about 4 minutes) for impurity F is about 1.6.

Inject reference solution (b). The resolution between methotrexate and impurity F should not be less than 2.0.

Inject reference solution (a). The area of peak due to impurity F not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (3.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 12.0 per cent, determined on 0.25 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 250.0 ml of the mobile phase.

Reference solution (a). Dissolve 25 mg of *methotrexate IPRS* in 250.0 ml of the mobile phase.

Reference solution (b). Dissolve 25 mg of *methotrexate IPRS* and 25 mg of *folic acid* in 250.0 ml of the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 92 volumes of *phosphate buffer pH 6.0* and 8 volumes of *acetonitrile*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b). The resolution between the peaks due to methotrexate and folic acid is not less than 5.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{20}H_{22}N_8O_5$.

Storage. Store protected from light and moisture.

CAUTION — Great care should be taken to prevent inhaling particles of Methotrexate and exposing the skin to it.

Methotrexate Injection

Methotrexate Injection is a sterile solution of Methotrexate in Water for Injections containing Sodium Hydroxide.

Methotrexate Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of methotrexate, $C_{20}H_{22}N_8O_5$.

Usual strengths. 2.5 mg per ml; 25 mg per ml.

Description. A clear, yellowish solution.

Identification

When examined in the range of 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at 258, 303 and 371 nm.

Tests

pH (2.4.24). 7.5 to 9.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection to obtain a solution containing 0.01 per cent w/v of Methotrexate in the mobile phase.

Reference solution (a). A 0.00002 per cent w/v solution of *methotrexate IPRS* in the mobile phase.

Reference solution (b). A 0.0003 per cent w/v solution of *methotrexate impurity C IPRS* in the mobile phase.

Reference solution (c). A solution containing 0.0003 per cent w/v each of *methotrexate IPRS* and *methotrexate impurity D IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS),
- mobile phase: a mixture of 7 volumes of *acetonitrile* and 93 volumes of a citro-phosphate buffer solution prepared by dissolving 7.8 g of *citric acid* and 17.9 g of *disodium hydrogen orthophosphate* in *water* and dilute to 1000 ml with *water*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Name	Relative retention time
Methotrexate impurity A[1]	0.2
Methotrexate impurity B[2]	0.3
Methotrexate impurity C[3]	0.4
Methotrexate impurity D[4]	0.8
Methotrexate	1.0
Methotrexate impurity E[5]	2.3

[1](2,4-diaminopteridin-6-yl)methanol,

[2] 4-aminofolic acid,

[3] *N*-methylfolic acid,

[4]4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]methylamino] benzoic acid,

[5]4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoic acid.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to methotrexate and methotrexate impurity D is not less than 2.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to methotrexate impurity C is not more that the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). The area of

any peak corresponding to methotrexate impurity B is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). The area of any peak corresponding to methotrexate impurity E is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of areas of any other secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.4 Endotoxin Unit per mg of methotrexate.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection with the mobile phase to produce a solution containing 0.0025 per cent w/v of Methotrexate.

Reference solution (a). A 0.0025 per cent w/v solution of *methotrexate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0025 per cent w/v each of *methotrexate IPRS* and *folic acid in* the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 92 volumes of *phosphate buffer pH 6.0* and 8 volumes of *acetonitrile*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject reference solution (b). The resolution between the peaks due to methotrexate and folic acid is not less than 5.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{20}H_{22}N_8O_5$ in the injection.

Storage. Store protected from light.

Labelling. The label states that the injection is not intended for intrathecal injection when an antimicrobial preservative is present.

Methotrexate Tablets

Methotrexate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methotrexate, $C_{20}H_{22}N_8O_5$.

Usual strength. 2.5 mg.

Identification

Extract a quantity of the powdered tablets containing 10 mg of Methotrexate with sufficient *0.1 M sodium hydroxide* to produce 100 ml, filter and dilute 10 ml of the filtrate to 100 ml with *0.1 M sodium hydroxide*.

When examined in the range 230 nm to 380 nm (2.4.7), the resulting solution shows absorption maxima at about 258 nm, 303 nm and 371 nm.

Tests

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute a suitable volume of the filtrate with the same solvent and measure the absorbance of the resulting solution at the maximum at about 306 nm (2.4.7). Calculate the content of $C_{20}H_{22}N_8O_5$ in the medium from the absorbance obtained from a solution of known concentration of *methotrexate IPRS*.

Q. Not less than 75 per cent of the stated amount of $C_{20}H_{22}N_8O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

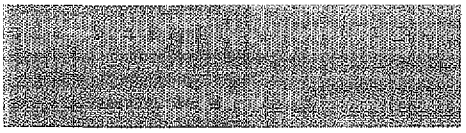
Test solution. Disperse a quantity of the powdered tablets containing about 10 mg of Methotrexate in 70 ml of the mobile phase with the aid of ultrasound for 5 minutes and dilute to 100.0 ml with the mobile phase, filter.

Reference solution (a). A 0.00002 per cent w/v solution of *methotrexate IPRS* in the mobile phase.

Reference solution (b). A 0.0003 per cent w/v solution of *methotrexate impurity C IPRS* in the mobile phase.

Reference solution (c). A solution containing 0.0003 per cent w/v each of *methotrexate IPRS* and *methotrexate impurity D IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS),
- mobile phase: a mixture of 7 volumes of *acetonitrile* and 93 volumes of a citro-phosphate buffer solution



prepared by dissolving 7.8 g of *citric acid* and 17.9 g of *disodium hydrogen orthophosphate*, dilute to 1000 ml with *water*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Name	Relative retention time
Methotrexate impurity A[1]	0.2
Methotrexate impurity B[2]	0.3
Methotrexate impurity C[3]	0.4
Methotrexate impurity D[4]	0.8
Methotrexate	1.0
Methotrexate impurity E[5]	2.3

[1](2,4-diaminopteridin-6-yl)methanol,

[2]4-aminofolic acid,

[3]*N*-methylfolic acid,

[4]4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]methylamino] benzoic acid,

[5] 4-[[(2,4-diaminopteridin-6-yl)methyl]methylamino]benzoic acid.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to methotrexate and methotrexate impurity D is not less than 2.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to methotrexate impurity C is not more than the area of the principal peak in the chromatogram obtained with solution (b) (3.0 per cent). The area of any peak corresponding to methotrexate impurity B is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of any peak corresponding to methotrexate impurity E is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of any other secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Carry out the test as described in the Assay, using the following solutions.

Test solution. Crush one tablet and mix with 100 ml of the mobile phase with the aid of ultrasound, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.0025 per cent w/v solution of *methotrexate IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0025 per cent w/v each of *methotrexate IPRS* and *folic acid* in the mobile phase.

Calculate the content of $C_{20}H_{22}N_8O_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 2.5 mg of Methotrexate and mix with 100.0 ml of the mobile phase with the aid of ultrasound, centrifuge and use the supernatant liquid.

Reference solution (a). A solution containing 0.0025 per cent w/v each of *methotrexate IPRS* and *folic acid* in the mobile phase.

Reference solution (b). A 0.0025 per cent w/v solution of *methotrexate IPRS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 92 volumes of *phosphate buffer pH 6.0* and 8 volumes of *acetonitrile*,
- flow rate: 1.4 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume: 20 µl.

Inject reference solution (a). The resolution between the peaks due to methotrexate and folic acid is not less than 5.0.

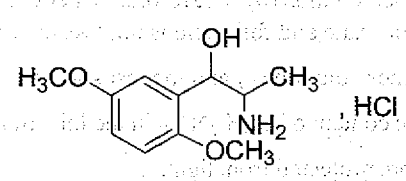
Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{20}H_{22}N_8O_5$ in the tablets.

Storage. Store protected from light and moisture.

Methoxamine Hydrochloride



$C_{11}H_{17}NO_3,HCl$ Mol. Wt. 247.7

Methoxamine Hydrochloride is *all-rac*-2-amino-1-(2,5-dimethoxyphenyl)propan-1-ol hydrochloride.

Methoxamine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{11}H_{17}NO_3$,HCl, calculated on the dried basis.

Category. Sympathomimetic.

Description. Colourless crystals or white, plate-like crystals or a white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methoxamine hydrochloride IPRS* or with the reference spectrum of methoxamone hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution shows an absorption maximum only at about 290 nm; absorbance at about 290 nm, about 0.69.

C. Dissolve 20 mg in 2 ml of *water*, add 5 ml of *diazotised nitroaniline solution* and 1 ml of *dilute sodium carbonate solution*. Allow to stand for 2 minutes and add 1 ml of *1 M sodium hydroxide*; a deep red colour is produced which is extractable with *1-butanol*.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.0, determined in a 2.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.14), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 86 volumes of *chloroform*, 12 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

Reference solution (a). A 0.02 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (b). A 0.01 per cent w/v solution of *2,5-dimethoxybenzaldehyde* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. Any spot corresponding to 2,5 dimethoxybenzaldehyde in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with a 0.3 per cent w/v solution of *ninhydrin* in *1-butanol* containing 3 per cent v/v of *glacial acetic acid* and heat at 105° for 5 minutes. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Dissolve 0.5 g in 30 ml of *anhydrous glacial acetic acid*, 15 ml of *mercuric acetate solution*, warming if necessary. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02477 g of $C_{11}H_{17}NO_3$,HCl.

Storage. Store protected from light and moisture.

Methoxamine Injection

Methoxamine Hydrochloride Injection

Methoxamine Injection is a sterile solution containing 2 per cent w/v of Methoxamine Hydrochloride in Water for Injections.

Methoxamine Injection contains not less than 1.90 per cent and not more than 2.10 per cent w/v of methoxamine hydrochloride, $C_{11}H_{17}NO_3$, HCl.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 290 nm.

B. Dilute 1 ml with 1 ml of *water*, add 5 ml of *diazotised nitroaniline solution* and 1 ml of *dilute sodium carbonate solution*. Allow to stand for 2 minutes and add 1 ml of *1 M sodium hydroxide*; a deep red colour is produced which is extractable with *1-butanol*.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 86 volumes of *chloroform*, 12 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

Test solution. Dilute the injection, if necessary, with *water* to contain 2.0 per cent w/v of Methoxamine Hydrochloride.

Reference solution (a). Dilute 1 volume of the test solution to 100 ml with *water*.

Reference solution (b). A 0.01 per cent w/v solution of *2,5-dimethoxybenzaldehyde* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 365 nm. Any spot corresponding to 2,5-dimethoxybenzaldehyde in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram

obtained with reference solution (b). Spray the plate with a 0.3 per cent w/v solution of *ninhydrin* in *1-butanol* containing 3 per cent v/v of *glacial acetic acid* and heat at 105° for 5 minutes. Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume containing about 100 mg of Methoxamine Hydrochloride add sufficient *water* to produce 100.0 ml. Dilute 5.0 ml of the solution to 100.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of $C_{11}H_{17}NO_3$,HCl taking 137 as the specific absorbance at 290 nm.

Storage. Store protected from light.

Industrial Methylated Spirit

IMS

Industrial Methylated Spirit is a mixture of nineteen volumes of Ethanol of an appropriate strength and one volume of approved wood naphtha.

Category. Pharmaceutical aid (solvent).

Description. Clear, colourless, mobile, volatile liquid.

Identification

Mix 0.1 ml with 0.05 ml of an 11 per cent w/w solution of *phosphoric acid* and 0.25 ml of *dilute potassium permanganate solution*. After 1 minute add a few mg of *sodium metabisulphite* and shake until the mixture is decolorised. Add 1.5 ml of a 50 per cent v/v solution of *sulphuric acid* and a few mg of finely powdered *chromotropic acid sodium salt*, shake well and heat on a water-bath for 5 minutes; a deep violet colour is produced.

Tests

Relative density (2.4.29). Not greater than 0.815.

Acidity or alkalinity. 25 ml requires not more than 0.2 ml of *0.1 M sodium hydroxide* to produce a pink colour with *phenolphthalein solution* and not more than 1.0 ml of *0.1 M hydrochloric acid* is required to produce a red colour with *methyl red solution*.

Appearance of solution. Dilute 5.0 ml to 100 ml with *water*; the solution is clear (2.4.1).

Aldehydes. Not more than 50 ppm, determined by the following method. To 5.0 ml add 5 ml of *water* and 1 ml of *decolourised fuchsin solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that obtained by treating in the same manner 5 ml of a 0.005 per cent w/v solution of redistilled *acetaldehyde* in *aldehyde-free ethanol (95 per cent)*.

Non-volatile matter. When evaporated and dried at 105°, leave not more than 0.01 per cent w/v of residue.

Storage. Store in tightly-closed containers at a temperature not exceeding 30°.

Labelling. The label states that it is inflammable.

Methylcellulose

Cellulose Methyl Ether

Methylcellulose is a cellulose having some of the hydroxyl groups in the form of the methyl ether. Various grades are available and are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/w solution measured at 20°.

Methylcellulose contains not less than 27.5 per cent and not more than 31.5 per cent of methoxyl (-OCH_3) groups, calculated on the dried basis.

Category. Bulk laxative; pharmaceutical aid (tablet excipient; suspending agent).

Description. A white or yellowish white or greyish white powder or granules; hygroscopic after drying.

Identification

A. With constant stirring add a quantity containing 1.0 g of the dried substance into 50 ml of *carbon dioxide-free water* previously heated to 90°. Allow to cool, dilute to 100 ml with *carbon dioxide-free water* and continue stirring until solution is complete (solution A). Heat 10 ml of solution A on a water-bath with stirring. At temperatures above 40° the solution becomes cloudy or a flocculent precipitate is formed. On cooling, the solution becomes clear.

B. To 10 ml of solution A add 0.3 ml of *2 M acetic acid* and 2.5 ml of a 10.0 per cent w/v solution of *tannic acid*; a yellowish white, flocculent precipitate is produced which dissolves in *6 M ammonia*.

C. Without heating completely dissolve 0.2 g in 15 ml of a 70 per cent w/w solution of *sulphuric acid*, pour the solution with stirring into 100 ml of iced *water*. In a test-tube kept in ice, mix thoroughly 1 ml of the solution with 8 ml of *sulphuric acid*, added dropwise. Heat in a water-bath for exactly 3 minutes and cool immediately in ice. When the mixture is cool, carefully add 0.6 ml of a solution containing 3 g of *ninhydrin* in 100 ml of a 4.55 per cent w/v solution of *sodium metabisulphite*, mix well and allow to stand at 25°; a pink colour is produced immediately which becomes violet within 100 minutes.

D. Place 1 ml of solution A on a glass plate. After evaporation of the *water* a thin film is produced.

Tests

Appearance of solution. Whilst stirring, introduce a quantity containing 1.0 g of the dried substance into 50 g of *carbon dioxide-free water* heated to 90°. Allow to cool, dilute to 100 g with the same solvent and continue stirring until solution is complete. Allow to stand at 2° to 8° for 1 hour. The resulting solution is not more opalescent than opalescence standard OS3 (2.4.1), and is not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 5.0 to 8.5, determined in solution A.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides (2.3.12). Dilute 5.0 ml of solution A to 15 ml with *water*. The resulting solution complies with the limit test for chlorides (0.5 per cent).

Apparent viscosity. Not less than 75 per cent and not more than 140 per cent of the declared value, determined by the following method. To 150 g of *water* heated to 90° add, with stirring, a quantity containing 6.0 g of the dried substance. Stir with a propeller-type stirrer for 10 minutes, place the flask in a bath of iced *water*, continue the stirring and allow to remain in the bath of iced *water* for 40 minutes to ensure that solution is complete. Adjust the weight of the solution to 300 g and centrifuge the solution to expel any trapped air. Determine the viscosity at 20° by Method C (2.4.28), using a shear rate of 10 s^{-1}.

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105°.

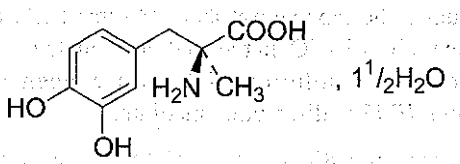
Assay. Weigh 50 mg in a hard gelatin capsule shell place the capsule and the contents in a 50-ml boiling flask and carry out the determination of methoxyl (2.3.29).

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.0005172 g of methoxyl ($-OCH_3$) groups.

Storage. Store protected from light and moisture.

Labelling. The label states the apparent viscosity in millipascal seconds of a 2 per cent w/w solution.

Methyldopa



$C_{10}H_{13}NO_4$, 1½ H_2O Mol. Wt. 238.2

Methyldopa is 3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine sesquihydrate.

Methyldopa contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{10}H_{13}NO_4$, calculated on the anhydrous basis.

Category. Antihypertensive.

Description. A white to yellowish white, fine powder which may contain friable lumps.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methyldopa IPRS* or with the reference spectrum of methyldopa.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.46.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose*.

Mobile phase. A mixture of 50 volumes of *1-butanol*, 25 volumes of *glacial acetic acid* and 25 volumes of *water*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *1 M hydrochloric acid*.

Reference solution. A 1 per cent w/v solution of *methyldopa IPRS* in *1 M hydrochloric acid*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of warm air, and spray with a solution freshly prepared by mixing equal volumes of a 10 per cent w/v solution of *ferric chloride* and a 5 per cent w/v solution of *potassium ferricyanide*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. To 10 mg add 3 drops of a 0.4 per cent w/v solution of *ninhydrin* in *sulphuric acid*; a dark purple colour is produced within 5 to 10 minutes. Add 0.15 ml of *water*; the colour changes to pale brownish yellow.

Tests

Appearance of solution. A 4.0 per cent w/v solution in *1 M hydrochloric acid* is not more intensely coloured than reference solution BYS6 or BS6 (2.4.1).

Acidity. Dissolve 1.0 g in 100 ml of *carbon dioxide-free water* with the aid of heat, add 0.15 ml of *methyl red solution* and titrate with *0.1 M sodium hydroxide*; not more than 0.5 ml is required to produce a pure yellow colour.

Optical rotation (2.4.22). −1.10° to −1.23°, determined in a solution prepared by dissolving a quantity containing 2.2 g of the anhydrous substance in 50.0 ml of *aluminium chloride solution*.

3-Methoxy compound and related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose*.

Mobile phase. A mixture of 65 volumes of *1-butanol*, 25 volumes of *water* and 15 volumes of *glacial acetic acid*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of a mixture of 96 volumes of *methanol* and 4 volumes of *7 M hydrochloric acid*.

Reference solution (a). A 0.005 per cent w/v solution of *3-methoxymethyldopa IPRS* in *methanol*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 10 µl of each of the test solution and reference solution (a) and 20 µl of reference solution (b). After development, dry the plate immediately in a current of warm air and spray with a mixture of 5 volumes of a 5 per cent w/v solution of *sodium nitrite* and 45 volumes of a 0.3 per cent w/v solution of *4-nitroaniline* in a mixture of 80 volumes of *hydrochloric acid* and 20 volumes of *water*. Dry it in a current of warm air and spray with a 20 per cent w/v solution of *sodium carbonate* and examine immediately. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Heavy metals (2.3.13). Dissolve 2.0 g in 10 ml of *water*, add 2 ml of *dilute acetic acid* and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 10.0 to 13.0 per cent, determined on 0.4 g.

Assay. Weigh 0.4 g and dissolve in 15 ml of *anhydrous formic acid*, 30 ml of *anhydrous glacial acetic acid* and 30 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02112 g of $C_{10}H_{13}NO_4$.

Storage. Store protected from light and moisture.

Methyldopa Tablets

Methyldopa Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of anhydrous methyldopa, $C_{10}H_{13}NO_4$. The tablets are coated.

Usual strengths. The equivalent of 125 mg, 250 mg and 500 mg of anhydrous methyldopa.

Identification

Remove the coating from a suitable quantity of the tablets by washing with *chloroform*. To a quantity of the powdered tablet cores containing 5 g of anhydrous methyldopa add 35 ml of a mixture of equal volumes of *chloroform* and *methanol* and shake for 3 minutes. Centrifuge and discard the supernatant liquid. Repeat the operation with a further 35 ml of a mixture of equal volumes of *chloroform* and *methanol*. Dry the residue in a current of nitrogen, add 20 ml of *methanol* and 15 ml of *2 M hydrochloric acid*, shake for 2 minutes and filter. Adjust the pH of the filtrate to 4.9 with *5 M ammonia*, allow to stand for several hours at 2° to 8° and filter. Wash the precipitate with 15 ml of *water* and dry it at 50° at a pressure not exceeding 0.7 kPa for 3 hours. Reserve a portion of the residue for the test for Specific optical rotation. The remainder of the residue complies with tests A and B.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methyldopa IPRS* or with the reference spectrum of methyldopa.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.46.

C. To a quantity of the powdered tablets containing 10 mg of anhydrous methyldopa, add 3 drops of a 0.4 per cent w/v solution of *ninhydrin* in *sulphuric acid*; a dark purple colour is produced within 5 to 10 minutes. Add 0.15 ml of *water*; the colour changes to pale brownish yellow.

D. To 10 mg of the powdered tablets add 2 ml of *0.1 M sulphuric acid*, 2 ml of *ferrous sulphate-citrate solution* and 0.5 ml of *dilute ammonia solution*; a dark purple colour is immediately produced.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, dilute suitably if necessary with the medium, at the maximum at about 280 nm (2.4.7). Calculate the content of $C_{10}H_{13}NO_4$ in the medium from the absorbance obtained from a solution of known concentration of *methyldopa IPRS* in dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{10}H_{13}NO_4$.

Optical rotation (2.4.22). – 0.98° to –1.09°, determined on a solution prepared by dissolving a weighed quantity of the residue obtained in the Identification test containing 0.39 g of $C_{10}H_{13}NO_4$ in sufficient *aluminium chloride solution* to produce 10.0 ml. The content of $C_{10}H_{13}NO_4$ in the residue used for the test may be determined by titrating with *0.1 M perchloric acid*, using 0.2 g of the residue, *crystal violet solution* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02112 g of $C_{10}H_{13}NO_4$.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of anhydrous methyldopa dissolve as completely as possible in sufficient *0.05M sulphuric acid* to produce 100 ml and filter. To 5 ml of the filtrate add 2 ml of *ferrous sulphate-citrate solution*, 8 ml of *glycine buffer solution* and sufficient *water* to produce 100 ml. Carry out the test simultaneously using 5 ml of a 0.11 per cent w/v solution of *methyldopa IPRS* in *0.05 M sulphuric acid*, instead of 5 ml of the filtrate, beginning with the words, " add 2 ml of *ferrous sulphate - citrate solution....*" Measure the absorbance of the test solution and the reference solution at about 545 nm (2.4.7) using as a blank solution, prepared by diluting 2 ml of *ferrous sulphate-citrate solution*, 8 ml of *glycine buffer solution* and sufficient *water* to produce 100 ml. Calculate the content of $C_{10}H_{13}NO_4$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous methyldopa.

Methyldopa and Hydrochlorothiazide Tablets

Methyldopa and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of methyldopa, $C_{10}H_{13}NO_4$ and hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

Usual strength. 250 mg methyldopa and 15 mg hydrochlorothiazide.

Identification

A. In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with the reference solution.

B. Shake a quantity of the powdered tablets containing 10 mg of methyldopa add 0.15 ml of solution of *ninhydrin* in *sulphuric acid* (1 volume in 250 volume). A dark purple colour is produced within 5 to 10 minutes. The colour changes to pale brownish yellow on adding of 0.15 ml of *water*.

Tests

Dissolution (2.5.2).

For Methyldopa —

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium. Reject the first few ml of the filtrate.

Reference solution. A 0.0275 per cent w/v solution of *methyldopa IPRS* in dissolution medium.

Solution A. A solution containing each of 1.0 per cent w/v solution of *ferrous sulphate*, 2.0 per cent w/v solution of *potassium sodium tartrate*, and 0.1 per cent w/v solution of *sodium bisulphite* in *water*.

Solution B. 5.0 per cent w/v solution of *ammonium acetate* in 20 per cent v/v *ethanol* and adjusted to pH 8.5 with *6 M ammonium hydroxide*.

Transfer an aliquot of the test solution containing 2-3 mg of methyldopa to a 100-ml volumetric flask. Adjust the final volume, if necessary, with medium to 100.0 ml. To a second 100-ml volumetric flask add 10.0 ml of reference solution, and to a third 100-ml volumetric flask add 10.0 ml of medium use as a blank. Pipette 5.0 ml of solution A into each flask, dilute with solution B to volume, and mix. measure the absorbance of the reference solution and the test solution at the wavelength of maximum at about 520 nm (2.4.7). Using the blank.

Q. Not less than 80 per cent of the stated amount of $C_{10}H_{13}NO_4$.

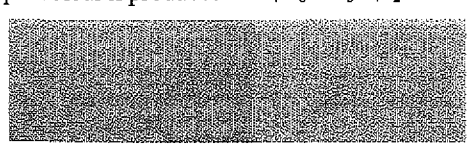
For Hydrochlorthiazide —

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 317 nm (2.4.7).

Calculate the content of hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$ in the medium from the absorbance obtained from a solution of known concentration of *hydrochlorothiazide IPRS* in dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_7H_8ClN_3O_4S_2$.



Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 250 mg of Methyldopa add 50.0 ml of *water,* 25.0 ml of *acetonitrile* and 13.0 ml of *1 M hydrochloric acid* and disperse with the aid of ultrasound for 5 minutes, cool, dilute to 250.0 ml with *water* and filter.

Reference solution. Dissolve suitable quantity of *methyldopa IPRS* to a volumetric flask to prepare 1 mg per ml solution and add a quantity of *hydrochlorthiazide IPRS* corresponds to the ratio of *hydrochlorthiazide* to *methyldopa* in a mixture of 1 volume of *water*, 1 volume of *acetonitrile*, and 0.5 volume of *1 M hydrochloric acid.* Dilute with *water* to volume.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 5 volumes of *methanol* and 95 volumes of a buffer solution prepared by dissolving 11.04 g of *monobasic sodium phosphate* in 1000 ml of *water*, adjusted to pH 2.8 with *orthophosphoric acid.*
- flow rate: 2 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the resolution between methyldopa and hydrochlorothiazide is not less than 6.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

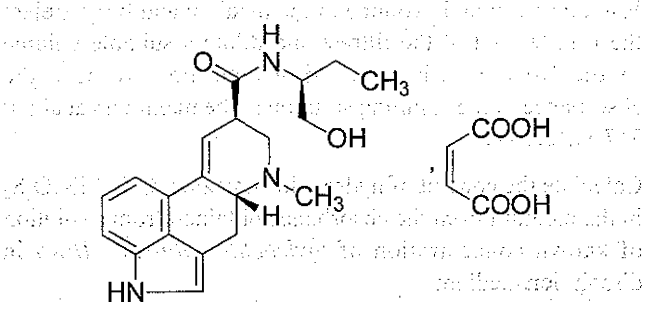
Inject the reference solution and the test solution.

Calculate the contents of $C_{10}H_{13}NO_4$ and $C_7H_8ClN_3O_4S_2$ in the tablets.

Storage. Store protected from moisture.

Methylergometrine Maleate

Methylergonovine Maleate



$C_{20}H_{25}N_3O_2,C_4H_4O_4$ Mol. Wt. 455.5

Methylergometrine Maleate is 9,10-didehydro-*N*-[(*S*)-1-(hydroxymethyl)propyl]-6-methylergoline-8β-carboxamide hydrogen maleate.

Methylergometrine Maleate contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{20}H_{25}N_3O_2,C_4H_4O_4$, calculated on the dried basis.

Category. Uterine stimulant.

Description. A white or faintly yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylergometrine maleate IPRS* or with the reference spectrum of methylergometrine maleate.

B. A 1.0 per cent w/v solution shows a blue fluorescence.

C. Dissolve 0.25 mg in 1 ml of *glacial acetic acid* containing a trace of *ferric chloride solution* and add carefully 1 ml of *sulphuric acid* and shake well; a deep blue colour is produced.

Tests

pH (2.4.24). 4.4 to 5.2, determined in a 0.02 per cent w/v solution.

Specific optical rotation (2.4.22). +44.0° to +50°, determined at 20° in a 0.5 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Carry out the test protected from light.

Test solution. Dissolve 25 mg of the substance under examination in 15 ml of mobile phase B and dilute to 50.0 ml with *water*.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with *water*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *water*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (3.5 µm),
- mobile phase: A. a 0.2 per cent w/v solution of *ammonium carbamate*,
  B. a mixture of equal volumes of *acetonitrile* and *water*,
- a gradient programme using the conditions given below,
- flow rate: 2 ml per minute,
- spectrophotometer set at 310 nm,
- injection volume: 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
2	85	15
7	65	35
12	65	35
17	20	80
19	20	80

Name	Relative retention time
Methylergometrine impurity A[1]	0.2
Methylergometrine impurity B[2]	0.5
Methylergometrine impurity C[3]	0.6
Methylergometrine impurity D[4]	0.7
Methylergometrine (Retention time: about 12 minutes)	1.0
Methylergometrine impurity I[5]	1.1
Methylergometrine impurity E[6]	1.14
Methylergometrine impurity F[7]	1.2
Methylergometrine impurity G[8]	1.3
Methylergometrine impurity H[9]	1.4

[1](6a*R*,9*R*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,

[2](6a*R*,9*S*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid,

[3](6a*R*,9*R*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide,

[4]ergometrine,

[5]1¢-*epi*-methylergometrine,

[6](6a*R*,9*S*)-7-methyl-4,6,6a,7,8,9-hexahydroindolo[4,3-*fg*]quinoline-9-carboxamide,

[7]ergometrinine,

[8]methysergide,

[9]methylergometrinine.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to methylergometrine impurity I is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of any peak corresponding to methylergometrine impurity C is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of any peak corresponding to methylergometrine impurities A, B, D, E, F, G and H is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying at 80° at a pressure not exceeding 2.7 kPa for 3 hours.

Assay. Weigh 20 mg and dissolve in sufficient *water* to produce 100.0 ml; dilute 20.0 ml of the solution to 100.0 ml with *water*. To 3.0 ml add 6.0 ml of *dimethylamino-benzaldehyde reagent*, mix, cool in running water for 5 minutes and add sufficient reagent to produce 10.0 ml. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination. Calculate the content of $C_{20}H_{25}N_3O_2$, $C_4H_4O_4$ from the absorbance obtained by repeating the Assay using *methylergometrine maleate IPRS* in place of the substance under examination.

Storage. Store protected from light, in an atmosphere of nitrogen, at a temperature between 2° to 8°.

Methylergometrine Injection

Methylergometrine Maleate Injection; Methylergonovine Maleate Injection; Methylergonovine Injection

Methylergometrine Injection is a sterile solution of Methylergometrine Maleate in Water for Injections free from dissolved air.

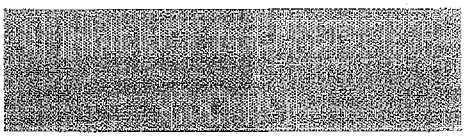
Methylergometrine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylergometrine maleate, $C_{20}H_{25}N_3O_2,C_4H_4O_4$.

Usual strength. 200 µg per ml.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

B. It exhibits a blue fluorescence.



C. To a volume containing 0.1 mg of Methylergometrine Maleate add 0.5 ml of *water* and 2 ml of *4-dimethylaminobenzaldehyde reagent*; after a few minutes a deep blue colour is produced.

Tests

pH (2.4.24). 2.7 to 3.5.

Related substances. *Protect the solutions from light throughout the test.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *chloroform* and 1 volume of *methanol*.

Test solution. Transfer a volume containing 1 mg of Methylergometrine Maleate to a separating funnel, add 1 ml of *sodium bicarbonate solution* and extract with three quantities, each of 5 ml, of *chloroform*. Evaporate the combined extracts to dryness at room temperature at a pressure not exceeding 0.7 kPa. Dissolve the residue in 0.25 ml of *methanol* and centrifuge, if necessary.

Reference solution (a). A 0.4 per cent w/v solution of *methylergometrine maleate IPRS* in *methanol*.

Reference solution (b). A 0.012 per cent w/v solution of *methylergometrine maleate IPRS* in *methanol*.

Place a beaker containing 25 ml of *strong ammonia solution* in the developing chamber, cover the chamber and allow to equilibrate for 30 minutes. Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Spray the plate with a solution containing 0.8 g of *4-dimethylaminobenzaldehyde* in a mixture of 90 ml of *ethanol* and 10 ml of *sulphuric acid*. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Bacterial endotoxins (2.2.3). Not more than 1.7 Endotoxin Units per µg of methylergometrine maleate.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *Protect the solutions from light throughout the Assay.*

To 1.0 ml add sufficient *water* to produce a solution containing 0.04 mg of Methylergometrine Maleate per ml. To 3.0 ml add 6.0 ml of *dimethylaminobenzaldehyde reagent*, mix, cool in running water for 5 minutes and add sufficient reagent to produce 10.0 ml. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination. Calculate the content of $C_{20}H_{25}N_3O_2,C_4H_4O_4$ from the absorbance obtained by repeating the Assay using *methylergometrine maleate IPRS* in place of the substance under examination.

Storage. Store protected from light, at a temperature between 2° to 8°.

Methylergometrine Tablets

Methylergometrine Maleate Tablets; Methylergonovine Maleate Tablets; Methylergonovine Tablets

Methylergometrine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylergometrine maleate, $C_{20}H_{25}N_3O_2,C_4H_4O_4$.

Usual strength. 125 µg.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

B. Extract a quantity of the powdered tablets containing 1 mg of Methylergometrine Maleate with 10 ml of *water*, filter and wash the residue with sufficient *water* to produce 10 ml; the solution has a blue fluorescence.

C. To 2 ml of the solution obtained in test B add 4 ml of *4-dimethylaminobenzaldehyde reagent*; a deep blue colour is produced after a few minutes.

Tests

Related substances. *Protect the solutions from light throughout the test.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *chloroform* and 1 volume of *methanol*.

Test solution. To a quantity of the powdered tablets containing 1 mg of Methylergometrine Maleate add 5 ml of *water*, 1 ml of *sodium bicarbonate solution* and 2 ml of *chloroform*. Shake, allow to separate and filter the chloroform layer through a plug of cotton moistened with *chloroform*. Repeat the extraction with a further 2 ml of *chloroform* and filter. Evaporate the combined extracts to dryness at room temperature at a pressure not exceeding 0.7 kPa, dissolve the residue in 0.25 ml of *methanol* and centrifuge, if necessary.

Reference solution (a). A 0.4 per cent w/v solution of *methylergometrine maleate IPRS* in *methanol*.

Reference solution (b). A 0.012 per cent w/v solution of *methylergometrine maleate IPRS* in *methanol*.

Place a beaker containing 25 ml of *strong ammonia solution* in the developing chamber, cover the chamber and allow to equilibrate for 30 minutes. Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Spray the plate with a solution containing 0.8 g of *4-dimethylaminobenzaldehyde* in a mixture of 90 ml of *ethanol* and 10 ml of *sulphuric acid*. Dry in a current of warm air for about 2 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. Complies with the test stated under Tablets.

Crush one tablet and transfer to a separating funnel with the aid of not more than 5 ml of *water* and add 3 ml of a 5 per cent w/v solution of *sodium carbonate*. Extract with four quantities, each of 5 ml, of *chloroform*. Filter the extracts through a plug of cotton moistened with *chloroform* into a 100-ml separating funnel. Add 2.0 ml of *water* and 10.0 ml of *4-dimethylamino-benzaldehyde reagent*.and shake vigorously for at least 90 seconds. Allow to stand for 30 minutes and discard the chloroform layer. Transfer the aqueous layer to a stoppered-tube and allow to stand for 60 minutes. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a mixture of 2.0 ml of *water* and 10.0 ml of *4-dimethylaminobenzaldehyde reagent*. Calculate the content of $C_{19}H_{23}N_3O_2,C_4H_4O_4$ in the tablet from the absorbance obtained by carrying out the following operation simul-taneously. Weigh 12 mg of *methylergometrine maleate IPRS* and dissolve in sufficient *water* to produce 200.0 ml. To 2.0 ml add 10.0 ml of *4-dimethylaminobenzaldehyde reagent*, mix and cool in running water for 5 minutes. Measure the absorbance of the resulting solution at the maximum at about 550 nm, using as the blank a mixture of 2.0 ml of *water* and 10.0 ml of *4-dimethylamino-benzaldehyde reagent*.

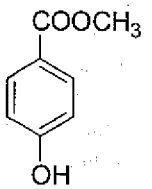
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 2 mg of Methylergo-metrine Maleate, dissolve in 50 ml of a 1 per cent w/v solution of *tartaric acid*. To 3.0 ml add 6.0 ml of *dimethylaminobenz-aldehyde reagent*, mix, cool in running water for 5 minutes and add sufficient reagent to produce 10.0 ml. Measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination. Calculate the content of $C_{20}H_{25}N_3O_2,C_4H_4O_4$ from the absorbance obtained by repeating the Assay using *methylergometrine maleate IPRS* in place of the substance under examination.

Storage. Store protected from light and moisture.

Methylparaben

Methyl Hydroxybenzoate



$C_8H_8O_3$ Mol. Wt. 152.2

Methylparaben is methyl 4-hydroxybenzoate.

Methylparaben contains not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_8O_3$.

Category. Pharmaceutical aid (antimicrobial preservative).

Description. A white or almost white, crystalline powder or colourless crystals.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylparaben IPRS* or with the reference spectrum of methylparaben.

B. Melting point (2.4.21). 125° to 128°.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity. Dissolve 1.0 g in sufficient *ethanol (95 per cent)* to produce 10 ml. To 2 ml of the solution add 3 ml of *ethanol (95 per cent)*, 5 ml of *carbon dioxide-free water* and 0.1 ml of *bromocresol green solution*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 2.5 ml of *methanol* and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 5 mg of *4-hydroxybenzoic acid* (methylparaben impurity A), and 5 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 50 mg of *methylparaben IPRS* in 2.5 ml of *methanol* and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of 0.68 per cent w/v solution of *potassium dihydrogen phosphate*, and 65 volumes of *methanol*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to methylparaben and methyl paraben impurity A is not less than 2.0. The relative retention time with reference to methyparaben for 4-hydroxybenzoic acid (methyparaben impurity A) is about 0.6.

Inject reference solution (c) and the test solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak due to methylparaben impurity A multiplied by 1.4 is not more than the area the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore the peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

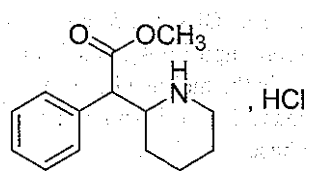
Assay. Determine by liquid chromatography (2.4.14), as described in the Related substances.

Inject reference solution (b) and the test solution.

Calculate the content of $C_8H_8O_3$.

Storage. Store protected from moisture.

Methylphenidate Hydrochloride



$C_{14}H_{19}NO_2,HCl$ Mol.Wt.269.8

Methylphenidate Hydrochloride is methyl α-phenyl-2-piperidineacetate hydrochloride.

Methylphenidate Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{14}H_{19}NO_2,HCl$, calculated on the dried basis.

Category. CNS stimulant.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylphenidate hydrochloride IPRS* or with the reference spectrum of methylphenidate hydrochloride.

B. It gives reaction (A) of chlorides (2.3.1).

Tests

Related substances. *NOTE — If ethylphenidate or bis-methylphenidate is a known process impurity, Procedure 2 is recommended.*

Method A

Determine by liquid chromatography (2.4.14) as described in the Assay with the following modifications.

Reference solution (a). A 0.0005 per cent w/v solution of *methylphenidate hydrochloride IPRS* in the mobile phase.

Name	Relative retention time
Erythro(R,S) isomer[1]	0.58
Methylphenidate impurity A[2]	0.85
Methylphenidate	1.0

[1]methyl (RS,SR)-2-phenyl-2-(piperidin-2-yl)acetate,

[2](RS,RS)2-Phenyl-2-(piperidin-2-yl)acetic acid.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to methylphenidate impurity A and methylphenidate is not less than 2.5 and the tailing factor is not more than 3.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to erythro(R,S) isomer is not more than the 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent). The area of any peak due to methylphenidate impurity A is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Method B

NOTE — Perform this test only if ethylphenidate or bis-1,2-(-carboxymethylbenzyl) piperidine is a known process impurity.

Test solution. Dissolve 5 mg of the substance under examination in mobile phase A and dilute to 10.0 ml with mobile phase A.

Reference solution (a). A 0.0005 per cent w/v solution of *methylphenidate hydrochloride IPRS* in mobile phase A.

Reference solution (b). A solution containing 0.05 per cent w/v of *methylphenidate hydrochloride IPRS*, 0.0003 per cent w/v each of *methylphenidate impurity A IPRS, phenylacetic acid IPRS, methylphenidate hydrochloride erythro isomer solution IPRS* in mobile phase A.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octcylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: A. a mixture of 7 volumes of *acetonitrile* and 43 volumes of solution prepared by adding 4 ml of *triethylamine* to 1000 ml of buffer solution prepared by dissolving 5.7 g of *monobasic ammonium phosphate* and 1.6 g of *1-octanesulphonate sodium* in 1000 ml of *water*, adjusted to pH 2.9 with *orthophosphoric acid*, B. a mixture of 4 volumes of *acetonitrile* and 1 volume of buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 2.8 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
7	65	35
10	50	50
12	50	50
13	90	10
16	90	10

Name	Relative retention time	Relative response factor
Methylphenidate impurity A[1]	0.55	1.1
Phenylacetic acid	0.67	1.0
Erythro(R,S) isomer[2]	0.8	1.0
Methylphenidate	1.0	—
Ethylphenidate[3]	1.22	0.9
Bis-methylphenidate[4]	1.8	2.6

[1](RS,SR)2-Phenyl-2-(piperidin-2-yl)acetic acid,

[2]methyl (RS,SR)-2-phenyl-2-(piperidin-2-yl)acetate,

[3]ethyl (RR,SS)-2-phenyl-2-(piperidin-2-yl)acetate,

[4]1,2-Bis(carboxymethylbenzyl)piperidine.

Inject reference solution (b). The test is not valid unless the resolution between methylphenidate impurity A and phenylacetic acid is not less than 2.7 and between phenylacetic acid and erythro isomer is not less than 3.6; the tailing factor for the methylphenidate peak is not more than 2.0 and the relative standard deviation for replicate injections for methylphenidate peak is not more than 2.0 per cent and not more than 5.0 per cent for methylphenidate impurity A , phenylacetic acid and methylphenidate hydrochloride erythroisomer.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak due to methylphenidate impurity A and erythro(R,S) isomer is not more than the 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in a vacuum at 60° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *methylphenidate hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.0005 per cent w/v of *methylphenidate impurity A IPRS* and 0.05 per cent w/v of *methylphenidate hydrochloride IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1 volume of *methanol* and 2 volumes of 0.27 per cent w/v solution of *monobasic potassium phosphate*, adjusted to pH 4.6 with *orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 209 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to methylphenidate impurity A and methylphenidate is not less than 2.5, the tailing factor is not more than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for the methylphenidate peak.

Inject reference solution (a) and the test solution. Run the chromatogram twice the retention time of the principal peak.

Calculate the content of $C_{14}H_{19}NO_2$,HCl.

Storage. Store protected from moisture.

Methylphenidate Hydrochloride Prolonged-release Tablets

Methylphenidate Hydrochloride Extended-release Tablets; Methylphenidate Hydrochloride Sustained-release Tablets

"*Methylphenidate Hydrochloride Prolonged-release Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the product for the different manufacturer may not be the same.*"

Methylphenidate Hydrochloride Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylphenidate hydrochloride, $C_{14}H_{19}NO_2$,HCl.

Usual strengths. 10 mg; 20 mg; 18 mg; 36 mg.

Identification

A. Place a portion of powdered tablets containing 100 mg of methylphenidate hydrochloride in a 100-ml beaker. Add 20 ml of *chloroform*, stir for 5 minutes, and filter, collecting the filtrate. Evaporate the filtrate to about 5 ml. Add *ethyl ether* slowly, with stirring, until crystals form. Filter the crystals, wash with *ethyl ether*, and dry at 80° for 30 minutes. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylphenidate hydrochloride IPRS* or with the reference spectrum of methylphenidate hydrochloride.

B. In the Related substances, methylphenidate hydrochloride peak in the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture A. 10 volumes of *acetonitrile* and 30 volumes of acidified *water*, adjusted to pH 3.0 with *orthophosphoric acid*.

Solvent mixture B. 10 volumes of *acetonitrile* and 10 volumes of *methanol*.

Test solution. Disperse a quantity of powdered tablets containing 10 mg of Methylphenidate Hydrochloride in solvent mixture B with the aid of ultrasound and dilute to 10.0 ml with solvent mixture B. Dilute 1.0 ml of the solution to 10.0 ml with solvent mixture A and centrifuge it.

Reference solution. A solution containing 0.00002 per cent w/v of *methylphenidate hydrochloride IPRS*, 0.00005 per cent w/v of methylphenidate hydrochloride erythro isomer from *methylphenidate hydrochloride erythro isomer solution IPRS* (this solution contains 0.5 mg of methylphenidate hydrochloride erythro isomer per ml in *methanol*) and 0.00015 per cent w/v of *methylphenidate impurity A IPRS* in solvent mixture A.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 2 g of *1-octanesulphonic acid sodium* in 730 ml of *water*, adjusted to pH 2.7 with *orthophosphoric acid*, mix with 270 ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 25 µl.

Name	Relative retention time
Methylphenidate impurity A[1]	0.47
Erythroisomer[2]	0.65
Methylphenidate hydrochloride	1.0

[1]α-phenyl-2-piperidineacetic acid,

[2]methyl (RS,SR)-2-phenyl-2-(piperidin-2-yl) acetate.

Inject the reference solution. The test is not valid unless the resolution between the methylphenidate and erythro isomer peaks is not less than 6.0 and the tailing factor for the methylphenidate peak is not more than 2.0.

Inject the refrence solution and the test solution. Run the chromatogram twice the retention time of the principal peak.

In the chromatogram obtained with the test solution, the area of any peak corresponding to Methylphenidate impurity A is not more than the area of the Methylphenidate impurity A peak in the chromatogram obtained with the reference solution (1.5 per cent). The area of any peak corresponding to erythroisomer is not more than the area of the methylphenidate

hydrochloride erythro isomer in the chromatogram obtained with the reference solution (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 12.5 times the area of the principal peak in the chromatogram obtained with the reference solution (2.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. A 0.04 per cent w/v solution of *phenylephrine hydrochloride* in the mobile phase.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of Methylphenidate Hydrochloride with 70 ml of the mobile phase with the aid of ultrasound for 15 minutes. Cool and dilute to 100.0 ml with the mobile phase, filter. To 10.0 ml of the solution, add 5.0 ml of the internal standard solution.

Reference solution. A 0.02 per cent w/v solution of *methylphenidate hydrochloride IPRS* in the mobile phase. To 10.0 ml of the solution, add 5.0 ml of the internal standard solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 40 volumes of *methanol*, 30 volumes of *acetonitrile* and 30 volumes of buffer solution prepared by dissolving 1.64 g of *anhydrous sodium acetate* in 900 ml of *water*, adjusted to pH 4.0 with *acetic acid* and dilute to 1000 ml with *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 50 µl.

The relative retention time with reference to methylphenidate hydrochloride for phenylephrine hydrochloride is about 0.8.

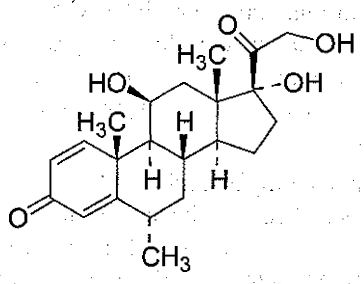
Inject the reference solution. The test is not valid unless the resolution between methylphenidate hydrochloride and internal standard peaks is not less than 2.0, the relative standard deviation from the peak response ratios of the methylphenidate hydrochloride to the internal standard is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{19}NO_2$,HCl in the tablets from the peak response ratios of methylphenidate hydrochloride to the internal standard obtained with the reference solution and the test solution respectively.

Storage. Store protected from moisture.

Methylprednisolone



$C_{22}H_{30}O_5$ Mol. Wt. 374.5

Methylprednisolone is 11β,17α,21-trihydroxy-6α-methylpregna-1,4-diene-3,20-dione.

Methylprednisolone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{22}H_{30}O_5$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylprednisolone IPRS* or with the reference spectrum of methylprednisolone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. *Chloroform*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *methylprednisolone IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*.

Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Dissolve about 2 mg in 2 ml of *sulphuric acid* by shaking and allow to stand for 5 minutes; an intense red colour is produced and the solution exhibits a reddish brown fluorescence when examined under ultraviolet light at 365 nm. Add the solution to 10 ml of *water* and mix; the colour fades and the solution exhibits a yellowish green fluorescence under ultraviolet light at 365 nm.

Tests

Specific optical rotation (2.4.22). +79.0° to +86.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 243 nm, 0.38 to 0.40.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in a mixture of equal volumes of *acetonitrile* and *methanol* and dilute to 10 ml with the solvent mixture.

Reference solution (a). Dissolve 2.0 mg of *methylprednisolone IPRS* and 2.0 mg of *betamethasone IPRS* in mobile phase A and dilute to 200 ml with mobile phase A.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with mobile phase A

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to silica gel (5 µm),
- column temperature: 45°,
- mobile phase A. a mixture of 250 volumes of *acetonitrile* and 700 volumes of *water* mixed, allowed to equilibrate and diluted to 1000 volumes with *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 2.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Time (min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
15	100	0
40	0	100
41	100	0
46	100	0

Equilibrate the column for at least 30 minutes with mobile phase B and then with mobile phase A for 5 minutes. For subsequent operations use the conditions described from 40 to 46 minutes.

Inject reference solution (a). When the chromatograms are recorded, the retention times are; methylprednisolone about 11.5 minutes, and betamethasone about 12.5 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone and betamethasone is at least 1.5; if necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not more than 0.5 the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak due to the blank and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh 0.1 g and dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml and mix. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol (95 per cent)* and mix well. Determine the absorbance of the resulting solution (2.4.7) at the maximum at about 243 nm. Calculate the content of $C_{22}H_{30}O_5$ taking 395 as the specific absorbance at 243 nm.

Storage. Store protected from light and moisture.

Methylprednisolone Tablets

Methylprednisolone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylprednisolone, $C_{22}H_{30}O_5$.

Usual strengths. 2 mg; 4 mg; 16 mg.

Identification

Extract a quantity of the powdered tablets containing 50 mg of Methylprednisolone with 100 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylprednisolone IPRS* or with the reference spectrum of methylprednisolone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

Test solution. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *methylprednisolone acetate IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of a layer of suitable thickness of the filtered solution at the maximum at about 246 nm (2.4.7). Calculate the content of $C_{22}H_{30}O_5$ in the medium taking 400 as the specific absorbance at 246 nm.

Q. Not less than 70 per cent of the stated amount of $C_{22}H_{30}O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A filtered mixture of 72 volumes of *water*, 25 volumes of *tetrahydrofuran* and 3 volumes of *glacial acetic acid*.

Test solution. Extract a quantity of the powdered tablets containing 25 mg of Methylprednisolone with the solvent mixture and dilute to 25 ml with the solvent mixture. Filter and centrifuge, if necessary.

Reference solution. A 0.001 per cent w/v solution of *methylprednisolone IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 149 volumes of *water*, 40 volumes of *tetrahydrofuran*, 10 volumes of *dimethylsulfoxide* and 1 volume of *butanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The column efficiency is not less than 800 theoretical plates and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Uniformity of content. Complies with the test stated under Tablets.

To one tablet add 0.5 ml of *water* (in the case of tablets containing 10 mg or less) or 1.0 ml of *water* (in the case of tablets containing more than 10 mg). Allow the tablet to stand for about 2 minutes, then swirl to disperse the tablet. Add 5.0 ml of the internal standard used in the assay for each mg of methylprednisolone, shake for 15 minutes, filter and centrifuge. Use the filtrate as the test solution.

Determine by liquid chromatography (2.4.14) using the chromatographic system and the reference solution described in the Assay.

Calculate the content of $C_{22}H_{30}O_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. Weigh a suitable quantity of prednisone in a 3 per cent v/v solution of *glacial acetic acid* in *chloroform* to obtain a solution having a known concentration of about 0.2 mg per ml of prednisone.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 10 mg of Methylprednisolone transfer to a suitable container and add 2.5 ml of *water*. Swirl to form a slurry. Add 50.0 ml of the internal standard solution, and shake for 15 minutes. Filter and centrifuge a portion of the filtrate if necessary and use this as the test solution.

Reference solution. Weigh a suitable quantity of *methylprednisolone IPRS* in the internal standard solution to obtain a solution having a known concentration of about 0.2 mg per ml of methylprednisolone.

Chromatographic system
- stainless steel column 25 cm x 4 mm, packed with porous silica particles (3 to 10 µm),
- mobile phase: a mixture of 475 volumes of *butyl chloride*, 475 volumes of *water-saturated butyl chloride*, 70 volumes of *tetrahydrofuran*, 35 volumes of *methanol*, and 30 volumes of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

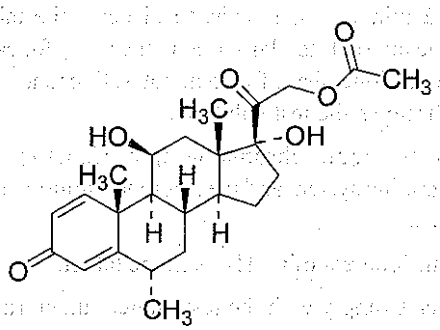
Inject the reference solution. The resolution between methylprednisolone and prednisone is not less than 4.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. The relative retention time with reference to methylprednisolone for prednisone is about 0.7.

Calculate the content of $C_{22}H_{30}O_5$ in the tablets.

Storage. Store protected from light and moisture.

Methylprednisolone Acetate



$C_{24}H_{32}O_6$ Mol. Wt. 416.5

Methylprednisolone Acetate is 11β,17α-dihydroxy-6α-methyl-3, 20-dioxopregna-1,4-dien-21-yl acetate.

Methylprednisolone Acetate contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{24}H_{32}O_6$, calculated on the dried basis.

Category. Adrenocortical steroid.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylprednisolone acetate IPRS* or with the reference spectrum of methylprednisolone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

Test solution. Dissolve 25 mg of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *methylprednisolone acetate IPRS* in 10.0 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Specific optical rotation (2.4.22). +97.0° to +105°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 240 nm, 0.34 to 0.37. The ratio of the absorbance at the maximum at about 240 nm to that at about 263 nm is 1.50 to 1.70.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 5 ml of *tetrahydrofuran* and dilute to 10.0 ml with *water*.

Reference solution (a). Dissolve 4.0 mg of *methylprednisolone acetate IPRS* and 4.0 mg of *dexamethasone acetate IPRS* in the mobile phase and dilute to 20.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 255 volumes of *tetrahydrofuran* and 745 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Equilibrate the column with the mobile phase for about 45 minutes.

Inject reference solution (a). The retention times are: methylprednisolone acetate, about 43 minutes and dexamethasone acetate about 57 minutes. The test is not valid unless the resolution between the peaks corresponding to methylprednisolone acetate and dexamethasone acetate is not less than 6.5. If necessary, adjust the concentration of *water* in the mobile phase.

Inject reference solution (b) and the test solution. Continue the chromatography for 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh 0.1 g and dissolve in sufficient *ethanol* to produce 100.0 ml and mix. Dilute 1.0 ml of the solution to 100.0 ml with *ethanol* and mix well. Determine the absorbance of the resulting solution at the maximum at about 243 nm (2.4.7). Calculate the content of $C_{24}H_{32}O_6$, taking 355 as the specific absorbance at 243 nm.

Storage. Store protected from light and moisture.

Methylprednisolone Acetate Injection

Methylprednisolone Acetate Injection is a sterile suspension of Methylprednisolone Acetate in Water for Injections.

Methylprednisolone Acetate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methylprednisolone acetate, $C_{24}H_{32}O_6$.

Usual strength. 40 mg per ml.

Description. A white suspension which settles on standing but readily disperses on shaking. On examination under a microscope, the particles are seen to be crystalline and rarely exceed 20 µm in diameter.

Identification

Dilute a volume containing 0.1 g of Methylprednisolone Acetate to 5 ml with *water*, centrifuge and discard the supernatant liquid. Wash the residue with five quantities, each of 5 ml, of *water*, resuspending the residue in *water* each time. Centrifuge and discard the washings. The residue, after drying at 105° for 3 hours, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylprednisolone acetate IPRS* or with the reference spectrum of methylprednisolone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. A mixture of 30 volumes of *toluene* and 10 volumes of *chloroform*.

Test solution. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *methylprednisolone acetate IPRS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram

obtained with reference solution (b) appears as a single, compact spot.

Tests

pH (2.4.24). 3.0 to 7.0.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Solution A. Mix 0.12 g of *prednisone IPRS* (internal standard) with 0.6 ml of *glacial acetic acid*, slowly add *chloroform* with the aid of ultrasound, shake to dissolve and dilute with sufficient *chloroform* to produce 20 ml.

Test solution. Add 10 ml of solution A to a measured quantity of the injection containing about 40 mg of Methylprednisolone Acetate, add sufficient *chloroform* to produce 25.0 ml and shake for 5 minutes or until the aqueous layer is clear; to 4.0 ml of the chloroform layer, add 30 ml of chloroform and 0.4 g of *anhydrous sodium sulphate*, shake for 5 minutes, and use the clear solution.

Reference solution. Dissolve 20 mg of *methylprednisolone acetate IPRS* in 5 ml of solution A and add sufficient *chloroform* to produce 100.0 ml.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with silica gel for chromatography (5 to 10 µm),
- mobile phase: a mixture of 30 volumes of *glacial acetic acid* and 35 volumes of *methanol*, 75 volumes of *tetrahydrofuran*, 475 volumes of water-saturated *1-chlorobutane* and 475 volumes of *1-chlorobutane*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to methylprednisolone and the internal standard is not less than 2.5.

Inject the reference solution and the test solution.

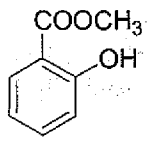
Calculate the content of $C_{24}H_{32}O_6$ in the injection from the peak response ratios of methylprednisolone to the internal standard obtained with the reference solution and the test solution respectively.

Storage. Store protected from light at a temperature not exceeding 30°. The injection should not be allowed to freeze.

Labelling. The label states (1) that the preparation is not to be given by intravenous injection; (2) that the container should be shaken gently before a dose is withdrawn.

Methyl Salicylate

Wintergreen Oil



$C_8H_8O_3$ Mol. Wt. 152.2

Methyl Salicylate is 2-hydroxybenzoic acid methyl ester.

Methyl Salicylate contains not less than 99.0 per cent w/w and not more than 100.5 per cent w/w of $C_8H_8O_3$.

Category. Counter-irritant.

Description. A colourless or slightly yellow liquid.

Identification

A. To 10 ml of a saturated aqueous solution add 0.05 ml of *ferric chloride test solution*; a violet colour develops.

B. Heat 0.25 ml with 2 ml of *2 M sodium hydroxide* on a water-bath for 5 minutes and add 3 ml of *1 M sulphuric acid*. Filter and wash the precipitate with *water*. The precipitate after drying at 105° for 1 hour melts at 156° to 161° (2.4.21).

Tests

Appearance of solution. To 2 ml add 10 ml of *ethanol (95 per cent)*. The resulting solution is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

Acidity. Dissolve 5.0 g in 50 ml of *ethanol (95 per cent)*, previously neutralised to a blue colour with *bromocresol green solution* by the addition of *0.1 M sodium hydroxide*. Not more than 0.4 ml of *0.1 M sodium hydroxide* is required to restore the blue colour.

Refractive index (2.4.27). 1.534 to 1.538.

Weight per ml (2.4.29). 1.175 g to 1.185 g.

Assay. Dissolve 0.5 g in 25 ml of *ethanol (95 per cent)*, add 0.05 ml of *phenol red solution* and neutralise with *0.1 M sodium hydroxide*. Add 50.0 ml of *0.1 M sodium hydroxide* and heat under a reflux condenser on a water-bath for 30 minutes. Cool and titrate with *0.1 M hydrochloric acid*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01522 g of $C_8H_8O_3$.

Storage. Store protected from light.

Methyl Salicylate Ointment

Strong Methyl Salicylate Ointment

Methyl Salicylate Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of methyl salicylate, $C_8H_8O_3$ in a suitable ointment base.

Usual strength. 10 per cent w/w.

Identification

In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Ointments.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). A solution of ointment containing 1.0 per cent w/v of Methyl Salicylate in *petroleum spirit (boiling range 80° to 100°)*.

Test solution (b). A solution of ointment containing 1.0 per cent w/v each of Methyl Salicylate and *benzyl alcohol* (internal standard) in *petroleum spirit (boiling range 80° to 100°)*.

Reference solution. A solution containing 1.0 per cent w/v of *benzyl alcohol* (internal standard) and 1.0 per cent w/v of *methyl salicylate IPRS* in *petroleum spirit (boiling range 80° to 100°)*.

Chromatographic system

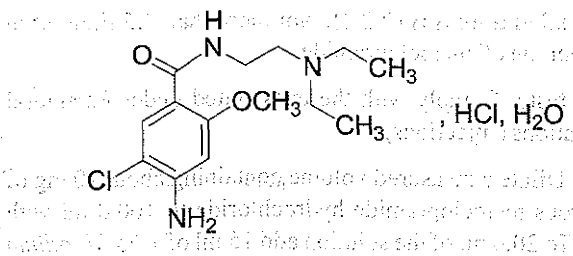
- a glass column 1.5m x 4.0 mm, packed with diatomaceous support (60 to 80 mesh), coated with 10.0 per cent w/v of polyethylene glycol 1540,
- temperature:
  column. 110°,
  inlet port and detector. 250°,
- flame ionization detector,
- flow rate: 60 ml per minute of the carrier gas.

Inject the reference solution and test solution (b).

Calculate the content of $C_8H_8O_3$ in the ointment.

Storage. Store protected from polystyrene plastic.

Metoclopramide Hydrochloride



$C_{14}H_{22}ClN_3O_2,HCl,H_2O$ Mol. Wt. 354.3

Metoclopramide Hydrochloride is 4-amino-5-chloro-*N*-(2-diethylaminoethyl)-2- methoxybenzamide hydrochloride monohydrate.

Metoclopramide Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{22}ClN_3O_2,HCl$, calculated on the anhydrous basis.

Category. Antiemetic.

Description. A white or almost white crystals or crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoclopramide hydrochloride IPRS* or with the reference spectrum of metoclopramide hydrochloride.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. A 5 per cent w/v solution in *carbon dioxide-free water* gives reaction (A) of chlorides (2.3.1).

D. Dissolve about 2 mg in 2 ml of *water*. The solution gives the reaction of primary aromatic amines (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 4.5 to 6.0, determined in a 10.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the substance under examination in the mobile phase to obtain a solution containing 0.1 per cent w/v of metoclopramide hydrochloride.

Reference solution (a). A 0.0005 per cent w/v solution of *metoclopramide hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 2.0 µg per ml each of *metoclopramide hydrochloride IPRS* and *metoclopramide impurity A (N-acetylmetoclopramide) IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 100 volumes of buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in

700 ml of *water*. Add 0.2 ml of *N,N-dimethyloctylamine*, adjusted to pH 4.0 with *orthophosphoric acid*, dilute to 1000 ml with *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to metoclopramide impurity A and metoclopramide is not less than 3.0.

Inject reference solution (a) and the test solution. Run the chromatogram 8 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Heavy metals (2.3.13). 12 ml of a 10 per cent w/v solution in *carbon dioxide-free water* complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (2 ppm Pb)* to prepare the standard.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 4.5 to 5.5 per cent, determined on 0.5 g.

Assay. Dissolve 0.25 g in a mixture of 50 ml of *ethanol (95 per cent)* and 5.0 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Note the volume added between the two inflections. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03363 g of $C_{14}H_{22}ClN_3O_2,HCl$.

Storage. Store protected from light and moisture.

Metoclopramide Injection

Metoclopramide Hydrochloride Injection

Metoclopramide Injection is a sterile solution of Metoclopramide Hydrochloride in Water for Injections free from dissolved air. It contains suitable buffering and stabilising agents.

Metoclopramide Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2,HCl$.

Usual strength. The equivalent of 10 mg of anhydrous metoclopramide hydrochloride in 2 ml.

Description. A clear, colourless solution.

Identification

A. Dilute a volume containing 10 mg of anhydrous metoclopramide hydrochloride to 500 ml with *0.01 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 273 nm and 309 nm.

B. To a volume containing 50 mg of anhydrous metoclopramide hydrochloride add 5 ml of *water* and 5 ml of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *1 M hydrochloric acid*; a yellow-orange colour is produced.

C. It gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 3.0 to 5.0.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light.

Test solution. Dilute a volume of injection with the mobile phase to obtain a solution containing 0.1 per cent w/v of anhydrous metoclopramide hydrochloride.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- mobile phase: *0.01 M sodium hexanesulphonate* in a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 4.0 with *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent).

Bacterial endotoxins (2.2.3). Not more than 2.5 Endotoxin Units per mg of metoclopramide.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute a measured volume containing about 10 mg of anhydrous metoclopramide hydrochloride to 100.0 ml with *water*. To 20.0 ml of the solution add 15 ml of *1.25 M sodium hydroxide* and extract with three quantities, each of 30 ml, of

chloroform, dry each extract with *anhydrous sodium sulphate* and filter. Dilute the combined extracts to 100.0 ml with *chloroform* and mix. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of $C_{14}H_{22}ClN_3O_2$,HCl, taking 265 as the specific absorbance at 305 nm.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous metoclopramide hydrochloride in a suitable dose-volume.

Metoclopramide Syrup

Metoclopramide Hydrochloride Syrup

Metoclopramide Syrup contains Metoclopramide Hydrochloride in a suitable flavoured vehicle.

Metoclopramide Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2$,HCl.

Usual strength. The equivalent of 5 mg of anhydrous metoclopramide hydrochloride in 5 ml.

Identification

To 50 ml add *5 M sodium hydroxide* till the solution becomes alkaline and extract with three quantities, each of 40 ml, of *chloroform*, dry each extract with *anhydrous sodium sulphate*. Evaporate the combined extracts to dryness on a water-bath. The residue complies with the following tests.

A. Dissolve 10 mg of the residue in *0.01 M hydrochloric acid* and dilute to 500 ml with *0.01 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 273 nm and 309 nm.

B. To 25 mg of the residue add 2.5 ml of *water* and 2.5 ml of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *1 M hydrochloric acid*; a yellow-orange colour is produced.

C. Dissolve about 2 mg in 2 ml of *water*. The solution gives the reaction of primary aromatic amines (2.3.1).

Tests

pH (2.4.24). 2.0 to 4.0.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light.

Test solution. Disperse a quantity of the oral solution containing 5 mg of anhydrous metoclopramide hydrochloride with the mobile phase and dilute to 10.0 ml with the mobile phase and filter.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- mobile phase: *0.01 M sodium hexanesulphonate* in a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 4.0 with *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peaks with a retention time relative to the principal peak of 0.5 or less.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Dilute a measured volume containing about 10 mg of anhydrous metoclopramide hydrochloride to 100.0 ml with *water*. To 20.0 ml of the solution add 15 ml of *1.25 M sodium hydroxide* and extract with three quantities, each of 30 ml, of *chloroform*, dry each extract with *anhydrous sodium sulphate* and filter. Dilute the combined extracts to 100.0 ml with *chloroform* and mix. Measure the absorbance of the resulting solution at the maximum at about 305 nm (2.4.7). Calculate the content of $C_{14}H_{22}ClN_3O_2$,HCl, taking 265 as the specific absorbance at 305 nm.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous metoclopramide hydrochloride in a suitable dose-volume.

Metoclopramide Tablets

Metoclopramide Hydrochloride Tablets

Metoclopramide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous metoclopramide hydrochloride, $C_{14}H_{22}ClN_3O_2$,HCl.

Usual strength. The equivalent of 10 mg of anhydrous metoclopramide hydrochloride.

Identification

A. Shake a quantity of the powdered tablets containing 10 mg of anhydrous metoclopramide hydrochloride with 50 ml of *0.01 M hydrochloric acid* and heat at 70° for 15 minutes with frequent shaking. Cool, dilute to 100 ml with *0.01 M hydrochloric acid*, filter and dilute 10 ml of the filtrate to 50 ml with the same solvent.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 273 nm and 309 nm.

B. Shake a quantity of the powdered tablets containing 50 mg of anhydrous metoclopramide hydrochloride with 5 ml of *water*, filter and add to the filtrate 5 ml of a 1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in *1 M hydrochloric acid*; a yellow-orange colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, dilute suitably if necessary with the medium, at the maximum at about 309 nm (2.4.7). Calculate the content of $C_{14}H_{22}ClN_3O_2$ in the medium from the absorbance obtained from a solution of known concentration of *metoclopramide hydrochloride IPRS* in dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{14}H_{22}ClN_3O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Carry out the test protected from light.

Test solution. Disperse a quantity of the powdered tablets containing 0.1 g of anhydrous metoclopramide hydrochloride with 20 ml of *methanol* and dilute to 100.0 ml with the mobile phase and filter.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- mobile phase: *0.01 M sodium hexanesulphonate* in a mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*, adjusted to pH 4.0 with *glacial acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent).

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described in the Assay with the following modifications.

Test solution. Disperse one tablet in 30 ml of *water*, with the aid of ultrasound for 20 minutes and dilute to 100.0 ml with *water*. Centrifuge and use the supernatant liquid.

Calculate the content of $C_{14}H_{22}ClN_3O_2$, HCl in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 20 mg of anhydrous metoclopramide hydrochloride with 80 ml of *water* with the aid of ultrasound for 10 minutes and then continue shaking mechanically for 20 minutes and dilute to 200.0 ml with *water*, filter.

Reference solution. A 0.01 per cent w/v solution of *metoclopramide hydrochloride IPRS* in *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Novapack C18),
- mobile phase: a mixture of 20 volumes of a solution prepared by dissolving 2.25 g of *sodium octanesulphonate* and 0.3 g of *sodium acetate* in sufficient *water* to produce 1000 ml and adjusted to pH 3.8 with *glacial acetic acid* and 80 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

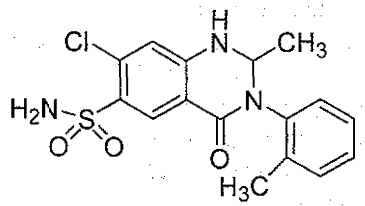
Inject the reference solution and the test solution.

Calculate the content of $C_{14}H_{22}ClN_3O_2$,HCl in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous metoclopramide hydrochloride.

Metolazone



$C_{16}H_{16}ClN_3O_3S$ Mol. Wt. 365.8

Metolazone is 7-chloro-1,2,3,4-tetrahydro-2-methyl-4-oxo-3-*o*-tolyl-6-quinazolinesulfonamide.

Metolazone contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{16}H_{16}ClN_3O_3S$, calculated on the dried basis.

Category. Diuretic.

Description. A white or slightly yellowish, crystalline powder. It shows polymorphism (2.5.11).

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metolazone IPRS* or with the reference spectrum of metolazone.

B. When examined in the range of 200 nm to 400 nm (2.4.7), a 0.0005 per cent w/v solution in *methanol* shows absorption maxima as obtained with *metolazone IPRS* of the same concentration.

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the substance under examination with *tetrahydrofuran* in 50 per cent of the total volume and dilute with *ethanol* to obtain a solution containing 0.06 per cent w/v of Metolazone.

Reference solution. A 0.048 per cent w/v solution of *metolazone IPRS* in *tetrahydrofuran*. Dilute a volume of the solution with *ethanol* to obtain a solution containing 0.0006 per cent w/v of Metolazone.

Use chromatographic system as described in the Assay with the following modification.

– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

Name	Relative retention time	Relative response factor
Desmethyl metolazone[1]	0.7	1.0
Metolazone benzamide analog[2]	0.8	0.83
Metolazone	1.0	1.0
meta-Metolazone[3]	1.3	0.91
para-Metolazone[4]	1.4	0.91
Didehydrometolazone[5]	1.5	0.83

[1] 7-chloro-2-methyl-4-oxo-3-phenyl-1,2,3,4-tetrahydroquinazoline-6-sulfonamide,

[2] 2-amino-4-chloro-5-sulfamoyl-*N*-(*o*-tolyl)benzamide,

[3] 7-chloro-2-methyl-4-oxo-3-(*m*-tolyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide,

[4] 7-chloro-2-methyl-4-oxo-3-(*p*-tolyl)-1,2,3,4-tetrahydroquinazoline-6-sulfonamide,

[5] 7-chloro-2-methyl-4-oxo-3-(*o*-tolyl)-3,4-dihydroquinazoline-6-sulfonamide.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.4 and the relative standard deviation is not more than 5.0.

Inject the reference solution and the test solution. Run the chromatogram 3.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.33 g complies with limit test for heavy metals, Method B (15 ppm).

Sulphated ash (2.4.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1 g by drying in an oven at 105° for 2 hours.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light.

Test solution. Dissolve a quantity of the substance under examination in *tetrahydrofuran* to obtain a solution containing 0.05 per cent w/v of Metolazone. Dilute a volume of the solution with *ethanol* to obtain a solution containing 0.005 per cent w/v of Metolazone.

Reference solution. A 0.05 per cent w/v solution of *metolazone IPRS* in *tetrahydrofuran*. Dilute a volume of the solution with *ethanol* to obtain a solution containing 0.005 per cent w/v of Metolazone.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile*, 25 volumes of *methanol* and 65 volumes of buffer solution prepared by dissolving 0.54 g of *monobasic potassium phosphate* in 100 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 15 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.4 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{16}ClN_3O_3S$.

Storage. Store protected from light and moisture.

Metolazone Tablets

Metolazone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metolazone, $C_{16}H_{16}ClN_3O_3S$.

Usual strengths. 2.5 mg; 5 mg; 10 mg.

Identification

When examined in the range 200 nm to 400 nm (2.4.7), a 0.00006 per cent w/v solution gives absorption maxima corresponds to that of the reference solution.

Tests

Dissolution (2.5.2).

NOTE — Protect the solutions from light.

Apparatus No. 2 (Paddle),
Medium. 900 ml of 2 per cent *sodium lauryl sulphate* in *0.05 M monobasic sodium phosphate*. Heat the mixture to about 37° to dissolve the *sodium lauryl sulphate* and adjusted to pH 7.5 with *10 M sodium hydroxide*,
Speed and time. 75 rpm and 120 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *metolazone IPRS* in *methanol* and dilute with dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 27 volumes of *acetonitrile*, 5 volumes of *methanol* and 68 volumes of *0.05 M monobasic potassium phosphate buffer*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Q. Not less than 75 per cent of the stated amount of $C_{16}H_{16}ClN_3O_3S$ in the tablet.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described in the Assay with the following modifications.

Test solution. Disperse 1 tablets in 0.5 ml of *water* and 10.0 ml of *methanol* with the aid of ultrasound for 30 minutes. If disintegration is not complete, sonicate for an additional 30 minutes. Shake by mechanical means for 30 minutes and dilute to 20.0 ml with *methanol*. Dilute a volume of the solution to obtain a solution containing 0.0005 per cent w/v of metolazone in the mobile phase.

Reference solution. A 0.025 per cent w/v solution of *metolazone IPRS* in *methanol*. Dilute a volume of the solution to obtain a solution containing 0.0005 per cent w/v solution of *metolazone IPRS* in mobile phase.

Calculate the content of $C_{16}H_{16}ClN_3O_3S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14.).

NOTE — Use low-actinic glassware throughout the *Assay.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 25 mg of *metolazone* in 70 ml *methanol* with the aid of ultrasound for 30 minutes, filter and dilute to 200.0 ml with *methanol*. Dilute a volume of the solution to obtain a solution containing 0.0005 per cent w/v of metolazone in mobile phase.

Reference solution. A 0.025 per cent w/v solution of *metolazone IPRS* in *methanol*. Dilute a volume of the solution to obtain a solution containing 0.0005 per cent w/v solution of *metolazone IPRS* in mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 28 volumes of *methanol*, 7 volumes of *acetonitrile* and 65 volumes of buffer solution prepared by dissolving 1.38 g of *monobasic potassium phosphate monohydrate* in 900 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*,
- flow rate: 1.1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 100 µl.

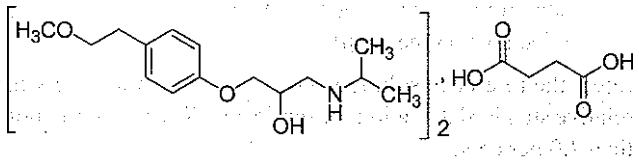
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate content of $C_{16}H_{16}ClN_3O_3S$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Metoprolol Succinate



$(C_{15}H_{25}NO_3)_2,C_4H_6O_4$ Mol. Wt. 652.8

Metoprolol Succinate is (*RS*)-1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol succinate.

Metoprolol Succinate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{15}H_{25}NO_3)_2,C_4H_6O_4$, calculated on the dried basis.

Category. Beta-adrenoceptor antagonist.

Description. A white, crystalline powder or colorless crystals.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoprolol succinate IPRS* treated in the same manner or with the reference spectrum of metoprolol.

Tests

pH (2.4.24). 7.0 to 7.6, determine in a 6.5 per cent w/v. solution in *carbon dioxide –free water*.

Related substances. Determine by liquid chromatography (2.4.14) as described in the Assay using the following modifications.

Test solution . Dissolve 50 mg of Metoprolol Succinate in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution. A 0.0001 per cent w/v solution of *metoprolol succinate IPRS* in the mobile phase.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak due to succinic acid.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.2 per cent, determined by drying in vaccum at 60° for 4 hours.

Assay. Determine by liquid chromatography(2.4.14)

Test solution. Dissolve 80 mg of Metoprolol Succinate in the mobile phase and dilute to 100.0 ml with the mobile phase.Dilute 5.0 ml of the solution to a 50.0 ml with the mobile phase.

Reference solution(a). A solution containing 0.0005 per cent each of *metoprolol succinate IPRS, metoprolol impurity A IPRS, metoprolol succinate B IPRS, metoprolol impurity C IPRS, metoprolol impurity D IPRS* in mobile phase.

Reference solution(b). A 0.008 per cent w/v solution of *metoprolol succinate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm×4 mm packed with octylsilane bonded to porous silica (3 to10 µm).
- mobile phase:a mixture of 60 volume of buffer prepared by dissolving 1.3 g of *sodium dodecyl sulphate* in 1000 ml of 0.1 per cent w/v *phosphoric acid* and 40 volume of *acetonitrile*,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 223 nm,
- injection volume: 10 µl.

Name	Relative retention time
Metoprolol impurity C[1]	0.6
Metoprolol impurity B[2]	0.7
Metoprolol impurity A[3]	0.8
Metoprolol succinate	1.0
Two diastereomers of Metoprolol Impurity D[4]	5.0 and 5.2

[1](±)4-[2-Hydroxy-3-(1-methylethyl)aminopropoxy]benzaldehyde,

[2](±)1-Chloro-2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propane,

[3](±)1-Ethylamino)-3-[4-(2-methoxyethyl)phenoxy]-propan-2-ol,

[4](±) N,N-Bis[2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl](1-methylethyl)amine.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to Metoprolol Impurity A and Metoprolol Impurity B is not less than 2.5 and the resolution between Metoprolol Impurity B and Metoprolol Impurity C is not less than 1.5.

Inject reference solution (b) the relative standard deviation of replicate injection is not more than 2.0 per cent.

Inject reference solution (b) and test solution.

Calculate the content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_4$.

Metoprolol Succinate Prolonged-release Tablets

Metoprolol Succinate Sustained-release Tablets; Metoprolol Succinate Extended-release tablets.

Metoprolol Succinate Extended Release Tablets manufactured by different manufacture, whilst complying with the requirements of monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Metoprolol Succinate Prolonged-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metoprolol succinate, $(C_{15}H_{25}NO_3)_2,C_4H_6O_4$.

Usual Strengths. 12.5 mg; 25 mg; 50 mg; 100 mg.

Identification

Extract a quantity of powdered tablets containing 200 mg of metoprolol succinate in a centrifuge tube with 40 ml of *phosphate buffer pH 6.8* and 40 ml of *dichloromethane* after shaking for 5 minutes. Extract 3 ml of the aqueous phase with 2 ml of *ammonium hydroxide* and 20 ml of *dichloromethane*. Collect and evaporate the *dichloromethane* phase, to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoprolol succinate IPRS* treated in the same manner or with the reference spectrum of metoprolol.

Tests

Dissolution (2.5.2). Complies with the test stated under Tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determined by liquid chromatography (2.4.14).

Test solution. Transfer 10 tablets in a 1000-ml volumetric flask. Add about 700 ml of 0.5M *methanolic hodrochloric acid* and shake for 15 minutes. Sonicate for 30 minutes with intermittent shaking. Dilute up to the mark with 0.5M *methanolic hydrochloric acid*. Filter through 0.45 µ membrane filter. Dilute the filtered solution quantitatively with 80 volume of *water* and 20 volume of *acetonitrile* to obtain a solution containing about 0.005 w/v of Metoprolol succinate.

Reference solution. A 0.05 w/v per cent solution of *Metoprolol succinate IPRS* in *0.5M methanolic hydrochloric acid*, Dilute 5.0 ml of the solution to 50.0 ml with the 80 volumes of *water* and 20 volumes of *acetonitrile*.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 250 ml of *acetonitrile* and 750 ml of buffer solution prepared by dissolving 50 ml of *1 M sodium dihydrogen orthophosphate* and 8.0 ml of *1 M orthophosphoric acid* diluted with *water* to 1000 ml and adjusted to pH 3.0 with *1 M potassium dihydrogen orthophosphate* or *1 M orthophosphoric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 40 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_4$ in the tablets.

Storage. Store at a temperature not exceeding 30°.

Labelling. Label it to indicate the content of metoprolol succinate and its equivalent, expressed as metoprolol tartrate.

Metoprolol Succinate Prolonged-release and Amlodipine Tablets

Metoprolol Succinate Prolonged-release and Amlodipine Besilate Tablets; Metoprolol Succinate Prolonged-release and Amlodipine Besylate Tablets.

Metoprolol Succinate Prolonged-release and Amlodipine besilate Tablets manufactured by different manufacturers, whilst complying with the requirements of the monograph, are not interchangeable, as the dissolution profile of the products of different manufacturers may not be the same.

Metoprolol Succinate Prolonged-release and Amlodipine Besilate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metoprolol succinate, $(C_{15}H_{25}NO_3)_2,C_4H_6O_4$ and amlodipine, $C_{20}H_{25}ClN_2O_5$.

Usual strengths. Metoprolol Succinate 47.5 mg and Amlodipine 5 mg; Metoprolol Succinate 23.75 mg and Amlodipine 5 mg; Metoprolol Succinate 23.75 mg and Amlodipine 2.5 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the principal peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

For Metoprolol Succinate. Complies with the test stated under Tablets.

For Amlodipine —

NOTE—Solutions use within 12 hours of preparation.

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.01M hydrochloric acid,*
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Use the chromatographic system as described under Assay with following modifications.

- spectrophotometer set at 239 nm,

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. Weigh accurately about 30.5 mg of *amlodipine besilate IPRS* into a volumetric flask, add about 5 ml of *methanol* and sonicate to dissolve, dilute to 200.0 ml with the dissolution medium. Dilute 5.0 ml of the solution to 200.0 ml with the dissolution medium and mix.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$.

Q. Not less than 70 per cent of the stated amount of $C_{20}H_{25}ClN_2O_5$.

Uniformity of content. Complies with the tests stated under Tablets.

For Amlodipine —

Determine by liquid chromatography (2.4.14), as described under Assay with following modification.

Test solution. Transfer one tablets into a volumetric flask and add about 5 ml of *acetonitrile* and sonicate to disperse the metoprolol part, add 20 ml of solvent mixture and sonicate for 10 minutes with constant shaking until the entire tablet gets dispersed. Further, add 20 ml of *acetonitrile* and sonicate for 5 minutes and add 20 ml of solvent mixture and sonicate for 15 minutes with constant shaking, allow the solution to cool to room temperature and dilute to 100.0 ml with the solvent mixture and mix. Centrifuge the solution at 3000 rpm for 10 minutes. Dilute the solution with solvent mixture to prepare *0.001 per cent w/v* of the amlodipine.

Reference solution. Weigh and transfer accurately about 27.8 mg of *amlodipine besilate IPRS* into a volumetric flask and dissolve in 100.0 ml of solvent mixture with the aid of ultrasound, dilute to volume with solvent mixture and mix. Further dilute 5.0 ml to 100.0 ml with solvent mixture and mix.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{25}ClN_2O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Throughout the entire sonication maintain the temperature of sonication bath below 20°. Use solution within 12 hours.

Solvent mixture. A mixture of 50 volumes of a buffer solution prepared by dissolving 7.0 g of *sodium dihydrogen orthophosphate monohydrate* in 1000 ml of *water,* adjusted to pH 3.0 with *orthophosphoric acid,* and 50 volumes of *acetonitrile.*

Test solution. Weigh and powder 20 tablets. Dissolve the quantity of the powder containing 20.0 mg of Amlodipine with about 25.0 ml of *acetonitrile* and sonicate for 5 minutes with intermittent shaking add 20.0 ml of solvent mixture and sonicate for 15 minutes with intermittent shaking and dilute to 50.0 ml with the same solvent. Allow to cool to room temperature.

Reference solution (a). A solution of *amlodipine besilate IPRS* containing 0.04 per cent w/v of amlodipine in the solvent mixture.

Reference solution (b). A 0.4 per cent w/v solution of *metoprolol succinate IPRS* in the solvent mixture.

Reference solution (c). Dilute reference solution (a) and (b) with the solvent mixture to get a 0.0004 per cent w/v solution of metoprolol succinate and 0.00004 per cent w/v solution of amlodipine.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5µm),
- sampler temperature: 10°,
- mobile phase A: a buffer solution prepared by Dissolving 7.0 g of *sodium dihydrogen orthophosphate*

monohydrate in 1000 ml of *water*, add 0.5 ml of *triethylamine* and mix thoroughly, adjusted to pH 4.5 with *orthophosphoric acid* and filter .

B: a mixture of 90 volumes of *methanol* and 10 volumes *tetrahydrofuran*.

- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
20	60	40
35	52	48
45	40	60
50	40	60
55	60	40
60	90	10
65	90	10

The relative retention time are about 0.65 for amlodipine impurity D and 1.0 for amlodipine and correction factor for amlodipine impurity D is 0.57.

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 1000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to amlodipine impurity D is not more than 10 times the area of the principal peak of amlodipine in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any other secondary peak is not more than 5 times the area of the principal peak of metoprolol in the chromatogram obtained with reference solution (c) (0.5 per cent) and the sum of the areas of all the secondary peaks excluding amlodipine impurity D is not more than 15 times the area of the principal peak of metoprolol in the chromatogram obtained with reference solution (c) (1.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak of metoprolol in the chromatogram obtained with the reference solution (c) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of a buffer solution prepared by dissolving 7.0 g of *sodium dihydrogen orthophosphate monohydrate* in 1000 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 50 volumes of *acetonitrile*.

Test solution. Weigh and transfer 5 tablets into a volumetric flask and add about 10 ml of *acetonitrile* and sonicate to disperse the Metoprolol part. Add 100 ml of a mixture of 30 volumes of *0.01M hydrochloric acid* and 70 volumes *acetonitrile* and sonicate for 10 minutes with constant shaking until the entire tablet gets dispersed. Further, add 100.0 ml of *acetonitrile* and sonicate for 5 minutes and add 100.0 ml of solvent mixture and sonicate for 15 minutes with constant shaking, allow the solution to cool to room temperature and dilute to volume to 500.0 ml with the solvent mixture and mix. Centrifuge the solution at 3000 rpm for 10 minutes. Dilute 5.0 ml of the solution to 25.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.05 per cent w/v of *metoprolol succinate IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.014 per cent w/v of *amlodipine besilate IPRS* in the solvent mixture with the aid of ultrasound.

Reference solution (c). Dilute reference solution (a) and (b) with the solvent mixture to obtain a solution having known concentration similar to the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 1 g of *1-deccane sulphonic acid* in 1000 ml *of water* and adjusted to pH 3.0 with *orthophosphoric acid,* and 40 volumes *acetonitrile*,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 20 µl.

Inject reference solution (c) The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

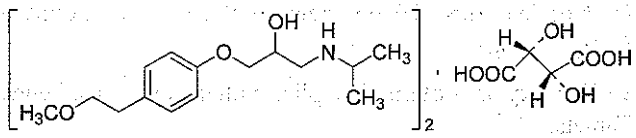
Inject reference solution (c) and the test solution.

Calculate the content of $(C_{15}H_{25}NO_3)_2$, $C_4H_6O_4$ $C_{20}H_{25}ClN_2O_5$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of amlodipine and metoprolol succinate and its equivalent expressed as metoprolol tartrate.

Metoprolol Tartrate



$(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ Mol. Wt. 684.8

Metoprolol Tartrate is (*RS*)-1-isopropylamino-3-*p*-(2-methoxyethyl)phenoxypropan-2-ol tartrate.

Metoprolol Tartrate contains not less than 99.0 per cent and not more than 101.0 per cent of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$, calculated on the dried basis.

Category. Beta-adrenoceptor antagonist.

Description. A white, crystalline powder or colourless crystals.

Identification

A.To 25 ml of a 0.4 per cent w/v solution add 2 ml of *5 M ammonia*, extract with 20 ml of *dichloromethane*, filter the lower layer through *anhydrous sodium sulphate* and evaporate to dryness. Place in a freezer for a few minutes to congeal the residue and allow to warm to room temperature.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoprolol tartrate IPRS* treated in the same manner or with the reference spectrum of metoprolol.

B. A 5 per cent w/v solution gives reaction (C) of tartrates (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution BS8 (2.4.1).

pH (2.4.24). 6.0 to 7.0, determined in a 2.0 per cent w/v solution.

Specific optical rotation (2.4.22). +7.0° to +10.0°, determined at 20° in a 2.0 per cent w/v solution.

Impurities M, N, O. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. Place 2 beakers, each containing 30 volumes of *concentrated ammonia*, at the bottom of a chromatographic tank containing a mixture of 20 volumes of *methanol* and 80 volumes of *ethyl acetate*.

Test solution. Dissolve 0.5 g of the substance under examination in *methanol* and dilute to 10.0 ml with *methanol*.

Reference solution (a). Dilute 1.0 ml of the test solution to 20.0 ml with *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with *methanol*.

Reference solution (b). Dilute 4.0 ml of reference solution (a) to 10.0 ml with *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air for atleast 3 hours and then expose the plate to iodine vapour for at least 15 hours. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent) and 1 such spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.003 per cent w/v of *metoprolol impurity A IPRS* and 0.005 per cent w/v of *metoprolol tartrate IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica with a pore size of 10 nm and a carbon loading of 19 per cent (5 µm),
- mobile phase: dissolve 3.9 g of *ammonium acetate* in 810 ml of *water*, add 2.0 ml of *triethylamine*, 3.0 ml of *orthophosphoric acid*, 10.0 ml of *glacial acetic acid* and 146 ml of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Metoprolol impurity H[1]	0.3	—
Metoprolol impurity C[2]	0.4	0.1
Metoprolol impurity G[3]	0.45	—
Metoprolol impurity F[4]	0.7	—
Metoprolol impurity A[5]	0.8	—
Metoprolol Tartrate (retention time: about 7 minutes)	1.0	—
Metoprolol impurity J[6]	1.4	—
Metoprolol impurity D[7]	1.6	—
Metoprolol impurity E[8]	1.8	—
Metoprolol impurity B[9]	2.0	—

[1](2*RS*)-1-[4-(2-hydroxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,

[2]4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy]benzaldehyde,

[3] 2-(4-hydroxyphenyl)ethanol,

[4] (2*RS*)-1-[(1-methylethyl)amino]-3-phenoxypropan-2-ol,

[5] (2*RS*)-1-(ethylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol,

[6]1-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol,

[7] (2*RS*)-3-[4-(2-methoxyethyl)phenoxy]propane-1,2-diol,

[8](2*RS*)-1-[2-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]propan-2-ol,

[9]4-(2-methoxyethyl)phenol.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to metoprolol impurity A and metoprolol is not less than 6.0.

Inject reference solution (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak due to metoprolol impurities A, B, C, D, E, F, G, H and J is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent). Ignore any peak due to tartaric acid.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.3 g in 30 ml *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03424 g of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$.

Storage. Store protected from light.

Metoprolol Injection

Metoprolol Tartrate Injection

Metoprolol Injection is a sterile solution of Metoprolol Tartrate in Water for Injections.

Metoprolol Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metoprolol tartrate, $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$.

Usual strength. 1 mg per ml.

Identification

D. To a volume of the injection containing 20 mg of Metoprolol Tartrate , add 2 ml of *5 M ammonia*, mix, extract with 30 ml of *dichloromethane*, filter the dichloromethane layer through *anhydrous sodium sulphate* and evaporate the filtrate to dryness using a rotary evaporator with gentle heating if necessary. Cool the residue to –18° for 30 minutes and allow to warm to room temperature. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoprolol tartrate IPRS*, treated in the same manner or with the reference spectrum of metoprolol.

B. In the test for Related substances, the principal peak in the chromatogram obtained with reference solution (a) corresponds to the peak in the chromatogram obtained with reference solution (b).

C. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection if necessary, with the mobile phase to obtain a solution containing 0.1 per cent w/v of Metoprolol Tartrate.

Reference solution (a). A 0.2 per cent w/v solution of *potassium sodium (+)-tartrate* in the mobile phase.

Reference solution (b). A mixture of 1 volume of reference solution (a) and 2 volumes of 0.005 per cent w/v solution of *fumaric acid* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with silica bonded to amine groups (10 µm) (Such as Lichrosorb NH_2),
- mobile phase: 1 per cent w/v *sodium chloride* in a mixture of 25 volumes of *methanol* and 75 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the chromatogram shows two clearly separated peaks.

Inject reference solution (a) and the test solution. The chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 5.5 to 7.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Evaporate a volume of the injection containing 25 mg of Metoprolol Tartrate to dryness at a temperature not exceeding 40° and dissolve the residue in 5.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Further dilute 3.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v of *metoprolol tartrate IPRS* and 0.003 per cent w/v of *metoprolol impurity A IPRS* in the mobile phase.

Reference solution (c). Prepare the solution in a fume cupboard in the following manner if necessary. Place an evaporating dish 10 cm in diameter containing 10 ml of a 0.1 per cent w/v solution of *metoprolol tartrate IPRS* in *0.1 M hydrochloric acid* so that the surface of the solution is 5 cm from a lamp emitting ultraviolet light at 254 nm for 6 hours. Dilute 1 volume of the solution to 50 volumes with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *glacial acetic acid* , 146 volumes of *acetonitrile* and 810 volumes of 0.48 per cent w/v of *ammonium acetate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time
Metoprolol impurity C[1]	0.3
Metoprolol impurity A[2]	0.7
Metoprolol (retention time: about 7 minutes)	1.0

[1]4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy] benzaldehyde,

[2](2*RS*)-1-(ethylamino)-3-[4-(2-methoxyethyl) phenoxy]propan-2-ol.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to metoprolol and metoprolol impurity A is not less than 6.0.

Inject reference solution (a) and the test solution. Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of the areas of all the secondary peaks is not more than 1.7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). If any of the above limits are exceeded, and if a secondary peak occurs with a retention time of 2 minutes (metoprolol impurity C), then in the chromatogram obtained with the test solution, divide the area of the peak corresponding to the principal peak in the chromatogram obtained with reference solution (c) (metoprolol impurity C) by 10; this divided area is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent); the sum of this divided area and the areas of any other secondary peaks is not more than 1.7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.17 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Bacterial endotoxins (2.2.3). Not more than 25.0 Endotoxin Units per mg of metoprolol tartrate.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute a volume of the injection containing 3 mg of Metoprolol Tartrate to 20 ml with *water* and measure the absorbance of the resulting solution at the maximum at 274 nm (2.4.7). Calculate the content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ from the absorbance obtained with a 0.015 per cent w/v solution of *metoprolol tartrate IPRS*.

Metoprolol Tablets

Metoprolol Tartrate Tablets

Metoprolol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metoprolol tartrate, $(C_{15}H_{25}NO_3)_2, C_4H_6O_6$.

Usual strengths. 50 mg; 100 mg.

Identification

Transfer a quantity of the powdered tablets containing about 40 mg of Metoprolol Tartrate to a separator, add 25 ml of *water* and 4 ml of *5 M ammonia*, extract with 20 ml of *dichloromethane*, filter the lower layer through *anhydrous sodium sulphate* and evaporate to dryness. Place in a freezer for a few minutes to congeal the residue and allow to warm to room temperature.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoprolol tartrate IPRS* treated in the same manner or with the reference spectrum of metoprolol.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of simulated gastric juice, artificial (without enzyme),
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the solution and filter. Dilute appropriately with the dissolution medium if necessary. Measure the absorbance (2.4.7) of the solution at the maximum at about 275 nm.

Calculate the content of $(C_{15}H_{25}NO_3)_2$, $C_4H_6O_6$ in the medium from the absorbance obtained from a solution of known concentration of *metoprolol tartrate IPRS*.

Q. Not less than 80 per cent of the stated amount of $(C_{15}H_{25}NO_3)_2, C_4H_6O_6$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 0.5 g of Metoprolol Tartrate with 20 ml of *chloroform* for 10 minutes, centrifuge, remove and retain the supernatant layer. Extract the residue with a further 20 ml quantity of *chloroform*, centrifuge and remove the supernatant layer. Evaporate the combined chloroform extracts to dryness at ambient temperature, add sufficient mobile phase to produce 25 ml and mix. Dilute 5.0 ml of the solution to 20.0 ml with the mobile phase and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Further dilute 3.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v of *metoprolol tartrate IPRS* and 0.003 per cent w/v of *metoprolol impurity A IPRS* in the mobile phase.

Reference solution (c). Prepare the solution in the fuming cupboard by placing an evaporating dish 10 cm in a diameter containing 10 ml of a 0.1 per cent w/v solution of *metoprolol tartrate IPRS* in *0.1 M hydrochloric acid* so that the surface of the solution is 5 cm from a lamp emitting ultraviolet light at 254 nm for 6 hours. Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Symmetry C18),
- mobile phase: a mixture of 0.2 volume of *triethylamine*, 0.3 volume of *orthophosphoric acid*, 1 volume of *glacial acetic acid*, 14.6 volumes of *acetonitrile* and 81 volumes of a 0.39 per cent w/v solution of *ammonium acetate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Name	Relative retention time
Metoprolol impurity C[1]	0.3
Metoprolol impurity A[2]	0.7
Metoprolol tartrate (Retention itme: about 7 minutes)	1.0

[1]4-[(2*RS*)-2-hydroxy-3-[(1-methylethyl)amino]propoxy] benzaldehyde,

[2](2*RS*)-1-(ethylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to metoprolol and metoprolol impurity A is not less than 6.0.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of the areas of all the secondary peaks is not more than 1.7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

If any of the above limits are exceeded, and if a secondary peak occurs with a retention time of about 2 minutes (metoprolol impurity C), then in the chromatogram obtained with the test solution: divide the area of the peak corresponding to the principal peak in the chromatogram obtained with reference solution (c) (metoprolol impurity C) by 10: this divided area is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of this divided area and the areas of any other secondary peaks is not more than 1.7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Ignore any peak with an area less than 0.17 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.12 g of Metoprolol Tartrate, transfer to a 100-ml volumetric flask, add about 75 ml of *ethanol (95 per cent)* and shake for 15 minutes. Dilute to volume with *ethanol (95 per cent)*, mix and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of $(C_{15}H_{25}NO_3)_2$, $C_4H_6O_6$ from the absorbance obtained by repeating the operation using *metoprolol tartrate IPRS* in place of the substance under examination.

Metoprolol Tartrate and Hydrochlorothiazide Tablets

Metoprolol Tartrate and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metoprolol tartrate $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ and hydrochlorothiazide $C_7H_8ClN_3O_4S_2$.

Usual strengths. Metoprolol tartrate, 50 mg and Hydrochlorothiazide, 12.5 mg; Metoprolol tartrate, 25 mg and Hydrochlorothiazide, 12.5 mg.

Identification

A. Disperse a quantity of the powder tablets containing about 0.1 g of metoprolol tartrate with 30 ml of *0.1 M sodium*

hydroxide, shake for 20 minutes and dilute to 50.0 ml with *0.1 M sodium hydroxide,* mix. Filter a portion of the solution, discarding first few ml of the filtrate. Transfer 25 ml of the filtrate in to a separating funnel and extract with three quantities, each of 15 ml of *chloroform,* collect the chloroform layer and filter through *anhydrous sodium sulphate.* Evaporate the *chloroform* to dryness, and place in a freezer to form residue.

NOTE— Retain the aqueous layer remaining after extraction for identification test B.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metoprolol tartrate IPRS* treated in the same manner or with the reference spectrum of metoprolol.

B. Pass the aqueous layer from Identification test A through *0.1 M sodium hydroxide* prerinsed cotton. Dilute a portion of the filtrate quantitatively and stepwise with *0.1 M sodium hydroxide* to obtain a solution containing 0.001 per cent w/v of hydrochlorothiazide. The UV absorption spectrum of the solution exhibits maxima and minima at the same wavelengths as that of the reference solution prepared by dissolving 25 mg of *hydrochlorothiazide IPRS* in 50 ml of *0.1 M sodium hydroxide* in a separator, and extract with three 15-ml portions of *chloroform.* Discard the *chloroform* extracts, and pass the aqueous solution through *0.1 M sodium hydroxide* solution-prerinsed cotton. Dilute 2.0 ml of the filtrate to a 100.0 ml with *0.1 M sodium hydroxide* and mix.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of simultated gastric juice, artificial (without enzyme),
Speed and time. 100 rpm and 30 minutes.

For Metoprolol Tartrate —

NOTE— Retain about 30 ml of the remaining filtrate of the solution for the test of hydrochlorothiazide.

Withdraw about 125 ml of the solution under examination, allow to cool to room temperature, and filter, discarding the first few ml of the filtrate. Dilute suitably with medium to obtain a concentration of about 0.005 per cent w/v of metoprolol tartrate. Transfer to separate separators 50.0 ml of the filtrate, 50.0 ml of a solution of 0.005 per cent w/v of *metoprolol tartrate IPRS* and 50.0 ml of dissolution medium as a blank. Add 10 ml of *2.5 M sodium hydroxide* to each separator, and extract each with three 15 ml portions of *chloroform,* filtering the chloroform extracts through a plug of glass wool into individual 50-ml volumetric flasks. Dilute the contents of each flask with *chloroform* to volume, and mix. Measure the absorbance of the filtrate and the reference solution at the maximum at about 276 nm (2.4.7).

Calculate the content of $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$ in the medium.

For Hydrochlorothiazide —

Withdraw a suitable volume of the medium retained from the determination of metoprolol tartrate and filter through 0.8µm, discarding the first few ml of the filtrate to obtain 0.001 per cent w/v of hydrochlorothiazide. Measure the absorbance of the filtered solution and reference solution, suitably diluted with the medium to obtain 0.001 per cent w/v of *hydrochlorothiazide IPRS,* at the maximum at about 316 nm (2.4.7).

Calculate the content of $C_7H_8ClN_3O_4S_2$ in the medium.

Q. Not less than 80 per cent of the stated amounts of $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$ and $C_7H_8ClN_3O_4S_2$.

Diazotizable substances. Not more than 1.0 per cent, determined by the following method.

Test solution. Disperse a quantity of the powdered tablets containing 50 mg of hydrochlorothiazide in the mixture of 20 ml of *methanol* and 20 ml of *water.* Shake continuously for 5 to 10 minutes and dilute to 100.0 ml with the *water.*

Reference solution. Dissolve 5 mg of *benzothiadiazine impurity A (4-Amino-6-chloro-1,3-benzenedi-sulphonamide) IPRS* in 2.0 ml of *methanol* and dilute to 50.0 ml with *water.* Transfer 5.0 ml of the solution to 20 ml of *methanol* and dilute to 100.0 ml with *water.*

Transfer 5 ml each of the reference solution and the test solution into separate 50-ml volumetric flasks. Pipet 5 ml of *water* into a separate 50-ml volumetric flask as a blank, add 1 ml of *1.0 per cent w/v solution of sodium nitrite* and 5 ml of *10 per cent v/v solution of hydrochloric acid* in each flask and allow to stand for 5 minutes. Further add 2 ml of *2.0 per cent w/v solution of ammonium sulphamate* and allow to stand for 5 minutes with shaking then add 2 ml of *1.0 per cent w/v solution of freshly prepared disodium chromotropate* and 10 ml of 1.0 per cent w/v solution of *sodium acetate.* Dilute to volume with *water* and mix. Measure the absorbance of the resulting solution and the reference solution at about 500 nm (2.4.7).The absorbance of the test solution does not exceed with that of the reference solution .

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

For Metoprolol Tartrate —

Internal standard solution. A 0.036 per cent w/v solution of *oxprenolol hydrochloride IPRS* in the mobile phase.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 100 mg of Metoprolol Tartrate

in a 100-ml volumetric flask, add about 60 ml of *0.1M hydrochloric acid,* heat on a water-bath for 3 minutes, and sonicate for 5 minutes. Shake by mechanical means for 30 minutes. Allow the solution to cool at room temperature, dilute to volume with *0.1 M hydrochloric acid* and mix. Filter a portion of the solution, discarding the first few ml of the filtrate. Transfer 10.0 ml of the resulting solution to a separating funnel, add 2.0 ml of *2.5 M sodium hydroxide* and extract with three 25.0 ml portions of *chloroform.* Pass the chloroform extracts through *chloroform* prerinsed glass wool into a round-bottom flask, and evaporate on a rotary evaporator under vacuum to dryness. Add 20.0 ml of internal standard solution to the flask, sonicate for 3 minutes, and gently swirl to dissolve the residue in the flask. Filter a portion of the solution through a filter of 0.5 µm or finer porosity, discarding the first few ml of the filtrate and use the filtered solution.

Reference solution. Dissolve a quantity of *metoprolol tartrate IPRS* in *0.1 M hydrochloric acid* to obtain a solution containing 0.1 per cent w/v of metoprolol tartrate. Transfer 10.0 ml of the solution to a separating funnel, add 2.0 ml of *2.5 M sodium hydroxide*, and extract with three 25.0 ml portions of chloroform. Pass the chloroform extracts through *chloroform* prerinsed glass wool into a round-bottom flask, and evaporate on a rotary evaporator under vacuum to dryness. Add 20.0 ml of internal standard solution to the flask, sonicate for 3 minutes, and gently swirl to dissolve the residue in the flask.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution prepared by adding 0.961 g of *1-pentanesulphonic acid sodium monohydrate* and 82 mg of *anhydrous sodium acetate* to a mixture of 550 volumes of *methanol*, 450 volumes of *water* and 0.57 ml of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

The relative retention time for metoprolol tartrate is about 0.8 and for oxprenolol hydrochloride is about 1.0.

Inject the reference solution. The test is not valid unless the resolution between the metoprolol tartrate and oxprenolol hydrochloride is not less than 2.0 and the relative standard deviation is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$ in the tablets.

For Hydrochlorothiazide —

Internal standard solution. A 0.04 per cent w/v solution of *sulphanilamide IPRS* in *methanol.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 25 mg of Hydrochlorothiazide in a 50-ml volumetric flask, add about 10.0 ml of internal standard solution and 20 ml of *methanol*, sonicate for 5 minutes and shake by mechanical means for 30 minutes, dilute to volume with *methanol* and mix. Centrifuge a portion of the solution and filter the supernatant liquid through 0.5 µm filter. discarding the first few ml of the filtrate.

Reference solution (a). Dissolve a quantity of *benzothiadiazine impurity A IPRS* in internal standard solution to obtain a solution containing 0.1 per cent w/v of *benzothiadiazine impurity A IPRS.* Transfer 2.0 ml of the solution to a 10-ml volumetric flask and dilute to volume with the *methanol.*

Reference solution (b). Weigh about 50 mg of *hydrochlorothiazide IPRS in* 20.0 ml of internal standard solution and dilute to 100.0 ml with *methanol.*

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution prepared by dissolving 1.38 g of *monobasic sodium phosphate* in 780 ml of *water,* add 220 ml of *methanol* and mix,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 4 µl.

The relative retention time for sulphanilamide and benzothiadiazine impurity A are about 0.7 and 1.0 respectively.

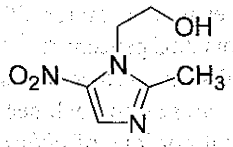
Inject reference solution (a) and (b). The test is not valid unless the resolution between *sulphanilamide IPRS* and *benzothiadiazine impurity A IPRS* is not less than 2.0 and the relative standard deviation is not more than 2.0 for reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_7H_8ClN_3O_4S_2$ in the tablets.

Storage. Store protected from light and moisture.

Metronidazole



$C_6H_9N_3O_3$ Mol. Wt. 171.2

Metronidazole is 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol.

Metronidazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_9N_3O_3$, calculated on the dried basis.

Category. Antiamoebic.

Description. A white or yellowish, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metronidazole IPRS* or with the reference spectrum of metronidazole.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 277 nm and a minimum at about 240 nm; absorbance at about 277 nm, between 0.365 and 0.395.

C. Heat about 10 mg in a water-bath with 10 mg of *zinc powder*, 1 ml of *water* and 0.25 ml of *2 M hydrochloric acid* for 5 minutes and cool. The solution gives the reaction of primary aromatic amines (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *1 M hydrochloric acid* is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution GYS4 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions protected from light.

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *2-methyl-4-nitroimidazole (metronidazole impurity A) IPRS* in the mobile phase, add 10.0 ml of the test solution and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture 30 volumes of *methanol* and 70 volumes of a 0.14 per cent w/v solution of *potassium dihydrogen phosphate,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 315 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to metronidazole and metronidazole impurity A is not less than 2.0.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent), the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

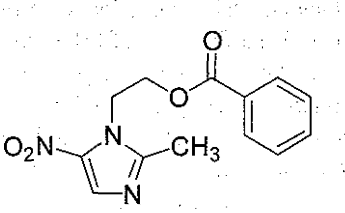
Assay. Dissolve 0.15 g in 50 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01712 g of $C_6H_9N_3O_3$.

Storage. Store protected from light and moisture.

Metronidazole Benzoate

Benzoylmetronidazole



$C_{13}H_{13}N_3O_4$ Mol. Wt. 275.3

Metronidazole Benzoate is 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl benzoate.

Metronidazole Benzoate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{13}H_{13}N_3O_4$, calculated on the dried basis.

Category. Antiamoebic; antiprotozoal; antibacterial.

Description. A white or cream-coloured, crystalline powder or flakes.

Indentification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol* shows an absorption maximum only at about 309 nm; absorbance at about 309 nm, about 0.3.

B. It gives reaction (B) of benzoates (2.3.1).

C. Melting range 98° to 102° (2.4.21).

Tests

pH (2.4.24). 5.0 to 7.0, determined in a 2.0 per cent w/v suspension.

Free benzoic acid. Not more than 0.2 per cent, determined by the following method. Dissolve 0.5 g in 25 ml of *ethanol (95 per cent)* and titrate with *0.01 M sodium hydroxide* using *phenol red solution* as indicator. Carry out a blank titration.

1 ml of *0.01 M sodium hydroxide* is equivalent to 0.001221 g of $C_7H_6O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 45 volumes of mobile phase B and 55 volumes of mobile phase A.

Test solution. Dissolve 0.1 g of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with di-isobutyloctadecylsilane bonded to porous silica (5 µm) with a specific surface area of 180 m^2/g, a pore size of 8 nm and a carbon loading of 10 per cent,
- mobile phase: A. 0.15 per cent w/v solution of *potassium dihydrogen phosphate,* adjusted to pH 3.2 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
5	80	20
15	55	45
40	55	45
45	80	20

Name	Relative retention time
Metronidazole benzoate impurity A[1]	0.17
Metronidazole benzoate impurity B[2]	0.2
Metronidazole benzoate impurity C[3]	0.7
Metronidazole benzoate (Retention time: about 20 minutes)	1.0

[1]2-methyl-4-nitroimidazole,
[2]metronidazole,
[3]benzoic acid.

Inject reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.01 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Dissolve 0.25 g in 50 ml of *acetone*. Add 10 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02753 g of $C_{13}H_{13}N_3O_4$.

Storage. Store protected from light and moisture.

Metronidazole Injection

Metronidazole Intravenous Infusion

Metronidazole Injection is a sterile isotonic solution of Metronidazole in Water for Injections. It may contain suitable buffering agents.

Metronidazole Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metronidazole, $C_6H_9N_3O_3$.

Usual strength. 5 mg per ml.

Description. An almost colourless to pale yellow solution.

Identification

A. Shake a volume of the injection containing about 0.1 g of Metronidazole with 9 g of *sodium chloride* for 5 minutes. Add 20 ml of *acetone*, shake for further 5 minutes and allow to separate. Evaporate the upper layer to dryness. On the residue, determine by infrared absorption spectrophotometery (2.4.6). Compare the spectrum with that obtained with *metronidazole IPRS* or with the reference spectrum of metronidazole.

B. Heat 2 ml of the injection in a water-bath for 5 minutes with 10 mg of *zinc powder* and 0.25 ml of *2 M hydrochloric acid* for 5 minutes and cool in ice. The solution gives the reaction of primary aromatic amines (2.3.1).

Tests

pH (2.4.24). 4.5 to 7.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing about 100 mg of metronidazole in 100 ml with the mobile phase.

Reference solution (a). A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole IPRS* in the mobile phase.

Reference solution (b). A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole IPRS* in the test solution.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS1),
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of a *0. 01 M potassium dihydrogen orthophosphate* prepared by dissolving 1.4 g of *potassium dihydrogen orthophosphate* with 1000 ml of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 315 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to metronidazole and *2-methyl-5-nitroimidazole* is not less than 2.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the peak due to *2-methyl-5-nitroimidazole* in the chromatogram obtained with reference solution (a) (0.5 per cent).

Bacterial endotoxins (2.2.3). Not more than 0.35 Endotoxin Unit per mg of metronidazole.

Other tests. Comply with the tests stated under Parenteral Preparations (Intravenous Infusions).

Assay. Dilute a suitable volume with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.001 per cent w/v of Metronidazole. Measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination.

Calculate the content of $C_6H_9N_3O_3$ from the absorbance obtained by repeating the operation using *metronidazole IPRS* in place of the substance under examination.

Storage. Store protected from light, in single dose containers.

Labelling. The label states that the contents should not be used if they contain any visible solid particles.

Metronidazole Gel

Metronidazole Gel contains Metronidazole in a suitable water-soluble basis. It may contain suitable preservatives.

Metronidazole Gel contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metronidazole, $C_6H_9N_3O_3$.

Usual strengths. 0.5 per cent w/w; 0.75 per cent w/w; 0.8 per cent w/w; 1 per cent w/w; 2 per cent w/w.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.0 to 6.5.

Other tests. Comply with the tests stated under Gels.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the gel equivalent to 75 mg of Metronidazole in a 100-ml volumetric flask, add 50 ml of mobile phase and shake on mechanical shaker for 20 minutes. Dilute with the mobile phase to volume, centrifuge and use the clear, Supernatant liquid for further dilution. Dilute 5.0 ml of the solution to 50 ml with mobile phase and mix.

Reference solution. A solution containing 0.0075 per cent w/v of *metronidazole IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of buffer solution prepared by dissolving 1.5 g of *potassium hydrogen orthophosphate* and 1.3 g of *sodium dihydrogen orthophosphate* in 350 ml of *water* and 65 volumes of *methanol*,

– flow rate: 1 ml per minute,
– spectrophotometer set at 254 nm,
– injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_6H_9N_3O_3$ in the gel.

Storage. Preserve in laminated collapsible tubes at a temperature not exceeding 30°.

Metronidazole Benzoate Oral Suspension

Benzoylmetronidazole Oral Suspension

Metronidazole Benzoate Oral Suspension is a suspension of Metronidazole Benzoate in a suitable aqueous vehicle. It may contain suitable colouring, flavouring, sweetening, buffering, suspending and antimicrobial agents.

Metronidazole Benzoate Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of metronidazole, $C_6H_9N_3O_3$.

Usual strengths. 40 mg per ml; 50 mg per ml.

Identification

Extract a quantity of the suspension containing 0.5 g of metronidazole with *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum only at about 309 nm; absorbance at about 309 nm, about 0.3.

B. It gives reaction (B) of benzoates (2.3.1).

Tests

Metronidazole. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the oral suspension containing 200 mg of metronidazole with 150 ml of *methanol* and dilute to 250.0 ml with *water* and centrifuge.

Reference solution. Dissolve 20 mg of *metronidazole IPRS* in 150 ml of *methanol* and dilute to 250.0 ml with *water*. Dilute 1.0 ml of the solution to 10.0 ml with *60 per cent methanol*.

Chromatographic system
– a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
– mobile phase: a mixture of 40 volumes of a 1.25 per cent w/v solution of *ammonium acetate*, adjusted to pH 7.0 with *dilute acetic acid* or *dilute ammonia* and 60 volumes of *methanol*,
– flow rate: 1 ml per minute,
– spectrophotometer set at 310 nm,
– injection volume: 20 µl.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to metronidazole is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the oral suspension containing 200 mg of metronidazole with 150 ml of *methanol* and dilute to 250.0 ml with *water* and centrifuge. Dilute 1.0 ml of the solution to 10.0 ml with *60 per cent methanol*.

Reference solution. Dissolve 62.5 mg of *metronidazole benzoate IPRS* in 1 ml of *dimethylformamide* and 30 ml of *methanol* and dilute to 50.0 ml with *water*. Dilute 1.0 ml of the solution to 10.0 ml with *60 per cent methanol*.

Use chromatographic system as described in the test for Metronidazole.

Inject the reference solution and the test solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_6H_9N_3O_3$, weight in volume.

Storage. store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of metronidazole in a suitable dose-volume.

Metronidazole Sterile Suspension

Metronidazole Sterile Suspension is a sterile suspension of Metronidazole in Water for Injections. The suspension is prepared by suspending Metronidazole for Injection in the requisite amount of Water for Injections immediately before use.

The sterile suspension complies with the tests stated under Parenteral Preparations.

Usual Strength. 200 mg per 5 ml.

Storage. The suspension should be used immediately after preparation but in any case within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

Metronidazole for Injection

Metronidazole for Injection is a sterile material consisting of Metronidazole with or without excipients. It is filled in a sealed container.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Metronidazole Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metronidazole, $C_6H_9N_3O_3$.

Identification

Shake a quantity of the powder containing 0.1 g of Metronidazole with 40 ml of *chloroform* for 15 minutes, filter and evaporate the filtrate to dryness. The residue complies with the following test. Determine by infrared spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metronidazole IPRS* or with the reference spectrum of metronidazole.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Determine the weight of the contents of 10 containers. Disperse a weighed quantity of the mixed contents of 10 containers containing about 0.1 g of Metronidazole with 40 ml of *chloroform*, with the aid of ultrasound for 15 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 100 ml of mobile phase.

Reference solution (a). A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole IPRS* in the mobile phase.

Reference solution (b). A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole IPRS* in the test solution.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of *0.01M potassium dihydrogen orthophosphate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 315 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to metronidazole and *2-methyl-5-nitroimidazole* is not less than 2.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the peak due to *2-methyl-5-nitroimidazole* in the chromatogram obtained with reference solution (a) (0.5 per cent).

Assay. Determine the weight of the contents of 10 containers. Transfer a weighed quantity of the mixed contents of the 10 containers containing about 50 mg of Metronidazole, dissolve in 100 ml of *0.1 M hydrochloric acid*. Dilute 10 ml of the solution to 250 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7). Calculate the content of $C_6H_9N_3O_3$ taking 375 as the specific absorbance at 277 nm.

Storage. Store protected from light.

Metronidazole Tablets

Metronidazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metronidazole, $C_6H_9N_3O_3$.

Usual strengths. 200 mg; 400 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Metronidazole with 40 ml of *chloroform* for 15 minutes, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *metronidazole IPRS* or with the reference spectrum of metronidazole.

B. Heat a quantity of the powdered tablets containing 10 mg of Metronidazole in a water-bath with 10 mg of *zinc powder*, 1 ml of *water* and 0.25 ml of *2 M hydrochloric acid* for 5 minutes and cool in ice. The solution gives the reaction of primary aromatic amines (2.3.1).

C. Shake a quantity of the powdered tablets containing about 0.2 g of Metronidazole with 4 ml of *0.5 M sulphuric acid* and filter. To the filtrate add 10 ml of *picric acid solution* and allow to stand for 1 hour; the precipitate, after washing with cold *water* under suction and drying at 105°, melts at about 150° (2.4.21).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter

not more than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7). Calculate the content of $C_6H_9N_3O_3$ from the absorbance obtained by repeating the operation using *metronidazole IPRS* instead of the substance under examination.

Q. Not less than 85 per cent of the stated amount of $C_6H_9N_3O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of powdered tablets containing about 100 mg of Metronidazole in 100 ml of the mobile phase,

Reference solution (a). A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole IPRS* in the mobile phase.

Reference solution (b). A 0.0005 per cent w/v solution of *2-methyl-5-nitroimidazole IPRS* in the test solution.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS1),
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of a *0.01 M potassium dihydrogen orthophosphate* prepared by dissolving 1.4 g of *potassium dihydrogen orthophosphate* with 1000 ml of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 315 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peak due to metronidazole and *2-methyl-5-nitroimidazole* is not less than 2.

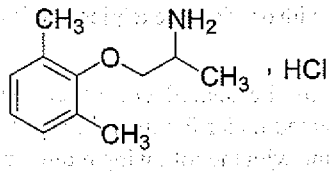
Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the peak due to *2-methyl-5-nitroimidazole* in the chromatogram obtained with reference solution (a).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.2 g of Metronidazole, transfer to a sintered-glass crucible and extract with six quantities, each of 10 ml, of hot *acetone*. Cool, add to the combined extracts 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, using 0.1 ml of a 1 per cent w/v solution of *brilliant green* in *anhydrous glacial acetic* acid as indicator to a yellowish-green end-point. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01712 g of $C_6H_9N_3O_3$.

Mexiletine Hydrochloride



$C_{11}H_{17}NO,HCl$ Mol. Wt. 215.7

Mexiletine Hydrochloride is (*RS*)-1-methyl-2-(2,6-xylyloxy) ethylamine hydrochloride.

Mexiletine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{11}H_{17}NO,HCl$, calculated on the anhydrous basis.

Category. Antiarrhythmic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mexiletine hydrochloride IPRS* or with the reference spectrum of mexiletine hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution in *0.01 M hydrochloric* acid shows an absorption maximum at about 260 nm; absorbance at 260 nm, about 0.46.

C. Dissolve 0.1 g in 3 ml of *0.02 M hydrochloric acid* and add a few crystals of *sodium nitrite*. Nitrogen is evolved and a yellow colour may be produced slowly.

D. It gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 4.0 to 5.5, determined in a 10.0 per cent w/v solution.

Impurity D. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel*.

Mobile phase. A mixture of 3 volumes of *ammonia*, 7 volumes of *ethanol (95 per cent)*, 45 volumes of *acetone* and 45 volumes of *toluene*.

Test solution. Dissolve 0.5 g of the substance under examination in *methanol* and dilute to 5.0 ml with *methanol*.

Reference solution (a). A 0.001 per cent w/v solution of *mexiletine impurity D IPRS ((2RS)-2-(2,6-dimethylphenoxy) propan-1-amine IPRS)* in *methanol*.

Reference solution (b). Dilute 1.0 ml of the test solution to 20.0 ml with *methanol.*

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 5.0 ml with *methanol.*

Reference solution (d). Dilute 1.0 ml of reference solution (a) to 5.0 ml with reference solution (b).

Apply to the plate 5 µl of reference solution (d), (e) and the test solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and spray with *ninhydrin solution* and heat at 105° for 15 minutes or until the spots appear. In the chromatogram obtained with the test solution, any spot corresponding to mexiletine impurity D is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent). The chromatogram obtained with reference solution (d) shows 2 clearly separated spots.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase.

Reference solution (b). A 0.001 per cent w/v solution of *mexiletine impurity C IPRS* in the mobile phase and transfer the solution to a volumetric flask containing 16 mg of *2,6-dimethyl-phenol* and dilute to 20.0 ml with the mobile phase. Mix 1.0 ml of the solution with 2.0 ml of reference solution (a) and dilute to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *methanol* and 35 volumes of a solution prepared by dissolving 11.5 g of *anhydrous sodium acetate* in 500 ml of *water*, add 3.2 ml of *glacial acetic acid*, mix and allow to cool, adjusting to pH 4.8 with *glacial acetic acid* and dilute to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 262 nm,
- injection volume: 20 µl.

Name	Relative retention time
Mexiletine impurity C[1]	0.7
Mexiletine (Retention time: about 4 minutes)	1.0
Mexiletine impurity A[2]	1.8

[1]1,1'-[(3,3',5,5'-tetramethylbiphenyl-4,4'-diyl)bisoxy]dipropan-2-amine,

[2]2,6-dimethylphenol.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to mexiletine impurity C and mexiletine is not less than 5.0.

Inject reference solution (b) and the test solution. Run the chromatogram 5.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to mexiletine impurity A is not more than 2.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any peak corresponding to mexiletine impurity C is not more than 20 times the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The area of any other secondarypeak is not more than 0.5 times the area of the peak due to mexiletine in the chromatogram obtained with reference solution (b) (0.1 per cent). the sum of areas of all the secondary peaks is not more than 2.5 times the area of the peak due to mexiletine in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.25 times the area of the peak due to mexiletine in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method C (10 ppm). Use 2 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.4.19). Not more than 0.5 per cent, determined on 5 g.

Assay. Dissolve 0.15 g in 50 ml of a mixture of equal volumes of *anhydrous glacial acetic acid* and *acetic anhydride*, add 10 ml of *mercuric acetate solution.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02157 g of $C_{11}H_{17}NO,HCl$.

Storage. Store protected from light and moisture.

Mexiletine Capsules

Mexiletine Hydrochloride Capsules

Mexiletine Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of mexiletine hydrochloride $C_{11}H_{17}NO,HCl$.

Usual strengths. 50 mg; 100 mg.

Identification

A. Shake a quantity of the contents of the capsules containing about 0.5 g of Mexiletine Hydrochloride with 10 ml of *methanol*, filter, evaporate to dryness and dry the residue at 105°.

When examined in the range 230 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution of the residue in *0.01 M hydrochloric acid* shows an absorption maximum at 260 nm.

B. Dissolve 0.1 g of the residue obtained in test A in 3 ml of *0.02 M hydrochloric acid* and add a few crystals of *sodium nitrite*; nitrogen is evolved and a yellow colour may be produced slowly.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

D. A 1 per cent w/v solution of the residue obtained in test A gives the reactions of chlorides (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *water;*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter with an average pore size of 1.0 µm. Measure the fluorescence intensities using the maximum excitation wavelength at about 265 nm and the maximum emission wavelength at about 295 nm (2.4.5). Calculate the content of $C_{11}H_{17}NO$, HCl by comparing the fluorescence intensities obtained with a standard solution of a known concentration of *mexiletine hydrochloride IPRS* in *water*.

Q. Not less than 80 per cent of the stated amount of $C_{11}H_{17}NO$, HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the contents of capsules containing 0.2 g of Mexiletine Hydrochloride with 10 ml of the mobile phase with the aid of ultrasound for 10 minutes, filter through a 0.4-µm glass microfibre filter (Whatman GF/C is suitable) and use the filtrate.

Reference solution (a). A 0.2 per cent w/v solution of *mexiletine hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v of *mexiletine impurity C IPRS* and 0.08 per cent w/v of *2,6-dimethylphenol* in the mobile phase. Mix 1.0 ml of the solution with 2.0 ml of reference solution (a) and dilute to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil),
- mobile phase: a mixture of 35 volumes of a solution prepared by dissolving 11.5 g of *anhydrous sodium acetate* in 500 ml of *water*, add 3.2 ml of *glacial acetic acid*, mix and allow to cool, adjusted to pH 4.8 with *glacial acetic acid* and dilute to 1000 ml with *water* and 65 volumes of *methanol*,
- flow rate: 1 ml per minute;
- spectrophotometer set at 262 nm,
- injection volume: 20 µl.

Name	Relative retention time
Mexiletine impurity C[1]	0.7
Mexiletine (Retention time: about 4 minutes)	1.0
Mexiletine impurity A[2]	1.8

[1] 1,1'-[(3,3',5,5'-tetramethylbiphenyl-4,4'-diyl)bisoxy]dipropan-2-amine,

[2] 2,6- dimethylphenol.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to mexiletine and mexiletine impurity C is not less than 5.0.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 5.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to mexiletine impurity A is not more than 2.5 times the area of the peak due to 2,6-dimethylphenol in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any peak corresponding to mexiletine impurity C is not more than 20 times the area of the peak due to mexiletine impurity C in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.005 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh a quantity of the mixed contents of 20 capsules containing about 0.05 g of Mexiletine Hydrochloride, mix with 50 ml of *0.01 M hydrochloride acid*, shake for 30 minutes, dilute to 100.0 ml with *0.01 M hydro-chloric acid* and centrifuge. Measure the absorbance of the supernatant liquid at the maximum at about 260 nm (2.4.7). Calculate the content of $C_{11}H_{17}NO$,HCl taking 11.6 as the specific absorbance at 260 nm.

Storage. Store protected from light.

Mexiletine Injection

Mexiletine Hydrochloride Injection

Mexiletine Injection is a sterile solution of Mexiletine Hydrochloride in Water for Injections.

Mexiletine Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of Mexiletine Hydrochloride $C_{11}H_{17}NO$, HCl.

Usual strength. 250 mg per 10 ml.

Identification

A. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

B. A volume containing 2.5 mg of Mexiletine Hydrochloride diluted to 2 ml gives reaction (A) of chlorides (2.3.1).

Tests

pH (2.4.24). 5.0 to 6.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection to obtain 2.0 per cent w/v of Mexiletine Hydrochloride in the mobile phase.

Reference solution (a). A 0.2 per cent w/v solution of *mexiletine hydrochloride IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v of *mexiletine impurity C IPRS* and 0.08 per cent w/v of *2,6-dimethylphenol* in the mobile phase. Mix 1.0 ml of the solution with 2.0 ml of reference solution (a) and dilute to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil),
- mobile phase: a mixture of 35 volumes of a solution prepared by dissolving 11.5 g of *anhydrous sodium acetate* in 500 ml of *water*, add 3.2 ml of *glacial acetic acid*, mix and allow to cool, adjusted to pH 4.8 with *glacial acetic acid* and dilute to 1000 ml with *water* and 65 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 262 nm,
- injection volume: 20 µl.

Name	Relative retention time
Mexiletine impurity C[1]	0.7
Mexiletine (Retention time: about 4 minutes)	1.0
Mexiletine impurity A[2]	1.8

[1]1,1'-[(3,3',5,5'-tetramethylbiphenyl-4,4'-diyl)bisoxy]dipropan-2-amine,

[2]2,6- dimethylphenol.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to mexiletine and mexiletine impurity C is not less than 5.0.

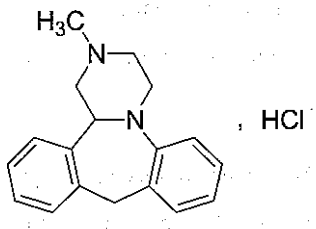
Inject reference solution (a), (b) and the test solution. Run the chromatogram 5.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to mexiletine impurity A is not more than 2.5 times the area of the peak due to 2,6-dimethylphenol in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any peak corresponding to mexiletine impurity C is not more than 20 times the area of the peak due to mexiletine impurity C in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. To a measured volume containing about 0.125 g of Mexiletine Hydrochloride add sufficient *0.01 M hydrochloric acid* to produce 250.0 ml and measure the absorbance of the resulting solution at the maximum at about 260 nm (2.4.7). Calculate the content of $C_{11}H_{17}NO$, HCl taking 11.6 as the specific absorbance at 260 nm.

Storage. Store in single dose containers.

Mianserin Hydrochloride



$C_{18}H_{20}N_2$,HCl Mol. Wt. 300.8

Mianserin Hydrochloride is (*RS*)-1,2,3,4,10, 14b-hexahydro-2-methyldibenzo[*c*,*f*] pyrazino[1,2-*a*]azepine hydrochloride.

Mianserin Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{20}N_2,HCl$, calculated on the dried basis.

Category. Antidepressant

Description. A white or almost white crystals or crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mianserin hydrochloride IPRS* or with the reference spectrum of mianserin hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution shows an absorption maximum only at about 279 nm; absorbance at about 279 nm, 0.64 to 0.72.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *cyclohexane*, 20 volumes of *ether* and 5 volumes of *diethylamine*.

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of *dichloromethane*.

Reference solution (a). A 0.2 per cent w/v solution of *mianserin hydrochloride IPRS* in *dichloromethane*.

Reference solution (b). A solution containing 0.2 per cent w/v, each of, *mianserin hydrochloride IPRS* and *cyproheptadine hydrochloride IPRS in dichloromethane*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of cold air for 5 minutes and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

D. It gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 5.5, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with endcapped octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 37 volumes of a buffer solution prepared by dissolving 5.0 g of *sodium octanesulphonate* in *water* and dilute to 350 ml with *water*, adjusted to pH 3.0 with a mixture of 1 ml of *orthophosphoric acid* and 3 ml of *water*, dilute to 400 ml with *water* and 63 volumes of *methanol*,
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Mianserin impurity B[1]	0.2	---
Mianserin impurity A[2]	0.5	2.4
Mianserin impurity D[3]	0.7	2.1
Mianserin (Retention time: about 18 minutes)	1.0	—
Mianserin impurity E[4]	1.1	—

[1](14b*RS*)-2-methyl-1,2,3,4,10,14b-hexahydrodibenzo[*c*,*f*]pyrazino[1,2-*a*]azepine-8-sulphonic acid,

[2][2-[(2*RS*)-4-methyl-2-phenylpiperazin-1-yl]phenyl]methanol,

[3][2-[(2*RS*)-4-benzyl-2-phenylpiperazin-1-yl]phenyl]methanol,

[4](14b*RS*)-1,2,3,4,10,14b-hexahydrodibenzo[*c*,*f*]pyrazino[1,2-*a*]azepine.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to mianserin impurity B is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent), the area of any peak corresponding to mianserin impurities A, D, E is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per

cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 65° over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 5 hours.

Assay. Weigh 0.2 g, dissolve in a mixture of 50 ml of *ethanol (95 per cent)* and 5 ml of *0.01 M hydrochloric acid*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Note the volume added between the two points of inflection.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03008 g of $C_{18}H_{20}N_2,HCl$.

Storage. Store protected from light and moisture.

Mianserin Tablets

Mianserin Hydrochloride Tablets

Mianserin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mianserin hydrochloride, $C_{18}H_{20}N_2$, HCl.

Usual strengths. 10 mg; 20 mg; 30 mg.

Identification

A. Shake a quantity of the powdered tablets containing about 20 mg of Mianserin Hydrochloride with 10 ml of *methanol*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mianserin hydrochloride IPRS* or with the reference spectrum of mianserin hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak due to mianserin in the chromatogram obtained with the reference solution.

C. The residue obtained in test A gives the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

Test solution. Triturate a quantity of the powdered tablets containing 40 mg of Mianserin Hydrochloride with 2 ml of a mixture of 4 volumes of *methanol* and 1 volume of *strong ammonia solution* and centrifuge.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with the same solvent mixture.

Reference solution (b). Dilute 1 volume of the test solution to 500 volumes with the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air for 5 minutes and expose to iodine vapour for 20 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 60 mg of Mianserin Hydrochloride with 30.0 ml of *0.2 M hydrochloric acid* for 1 hour and filter. To 10.0 ml of the filtrate add 3.0 ml of *1 M sodium hydroxide* and 10.0 ml of *toluene*, mix thoroughly, centrifuge and use the clear upper layer.

Test solution (b). To 10.0 ml of the filtrate obtained in test solution (a), add 3.0 ml of *1 M sodium hydroxide* and 10.0 ml of *toluene* containing 0.2 per cent w/v of *triphenylamine* (internal standard), mix thoroughly, centrifuge and use the clear upper layer.

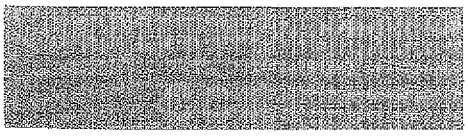
Reference solution. Add 3.0 ml of *1 M sodium hydroxide* and 10.0 ml of *toluene* containing 0.2 per cent w/v of *triphenylamine* (internal standard) to 10.0 ml of a solution containing 0.2 per cent w/v of *mianserin hydrochloride IPRS* in *0.2 M hydrochloric acid*, mix thoroughly, centrifuge and use the clear upper layer.

Chromatographic system
- a glass column 1.0 m × 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (Such as OV-17),
- temperature:
  column 255°,
  inlet port and detector at 240°,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

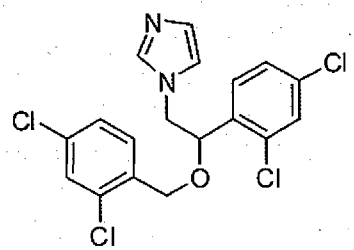
Inject 1 µl of test solution (a), (b) and the reference solution.

Calculate the content of $C_{18}H_{20}N_2$, HCl in the tablets.

Storage. Store protected from light and moisture.



Miconazole



$C_{18}H_{14}Cl_4N_2O$ Mol. Wt. 416.1

Miconazole is 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl-1*H*-imidazole.

Miconazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{18}H_{14}Cl_4N_2O$, calculated on the dried basis.

Category. Antifungal.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *miconazole IPRS* or with the reference spectrum of miconazole.

B. Determine by thin-layer chromatography (2.4.17), using *silica gel G*.

Mobile phase. A mixture of 20 volumes of *ammonium acetate solution,* 40 volumes of *dioxin* and 40 volumes of *methanol.*

Test solution. Dissolve 30 mg of the substance under examination in 5 ml of the mobile phase.

Reference solution (a). Dissolve 30 mg of *miconazole IPRS* in 5 ml of the mobile phase.

Reference solution (b). Dissolve 30 mg each of *miconazole IPRS* and *econazole nitrate IPRS* in 5 ml of the mobile phase.

Apply 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of warm air for 15 minutes. Expose to iodine vapour until the spots appear and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that obtained in the chromatogram with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two distinct spots.

C. To about 30 mg in a porcelain crucible add 0.3 g of *anhydrous sodium carbonate*. Heat on flame for 10 minutes, allow to cool. Take up the residue with 5 ml of *dilute nitric acid* and filter. To 1 ml of the filtrate add 1 ml of *water*. The solution gives reaction (A) of chlorides (2.3.1).

D. Melting range (2.4.21). 83° to 87°.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *methanol* (Solution A) is clear (2.4.1) and not more intensely coloured than the reference solution YS5 (2.4.1).

Optical rotation (2.4.22). – 0.10° to + 0.10°, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of the mobile phase.

Reference solution (a). Dissolve 2.5 mg each of *miconazole IPRS* and *econazole nitrate IPRS* in 100 ml of the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100 ml with the mobile phase. Dilute 5.0 ml of the solution to 20 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: dissolve 6.0 g of *ammonium acetate* in a mixture of 30 volumes of *acetonitrile,* 32 volumes of *methanol* and 38 volumes of *water,*
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to econazole and miconazole is not less than 10.

Inject reference solution (b) and the test solution. Run the chromatogram 1.2 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of secondary peak corresponding to (1*RS*)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol (miconazole impurity A), 1-[(2*RS*)-2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (miconazole impurity B), (2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl) ethanamine (miconazole impurity C), 1-[(2*RS*)-2-[(2,6-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (miconazole impurity D), 2-[1-[(2*RS*)-2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazol-3-io]-2-methylpropanoate (miconazole impurity E), 1-[(2*RS*)-2-[(3,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (miconazole impurity F), and 1-[(2*RS*)-2-[(2,5-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole (miconazole impurity G) is not more than the area of the principal peak in the chromatogram obtained with reference

solution (b) (0.25 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

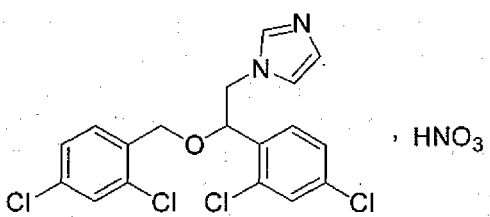
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 60° for 4 hours.

Assay. Weigh 0.3 g, dissolve in 50 ml of a mixture of 1 volume of *anhydrous acetic acid* and 7 volumes of *methyl ethyl ketone*. Titrate with *0. 1 M perchloric acid* using 0.2 ml of *naphtholbenzein solution* as indicator, until the colour changes from orange-yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04161 g of $C_{18}H_{14}Cl_4N_2O$.

Storage. Store protected from light.

Miconazole Nitrate



$C_{18}H_{14}Cl_4N_2O,HNO_3$ Mol. Wt. 479.2

Miconazole Nitrate is (*RS*)-1-[2-(2,4-dichlorophenylmethoxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate.

Miconazole Nitrate contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{18}H_{14}Cl_4N_2O,HNO_3$, calculated on the dried basis.

Category. Antifungal.

Description. A white or almost white, crystalline or microcrystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *miconazole nitrate IPRS* or with the reference spectrum of miconazole nitrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.04 per cent w/v solution in a mixture of 90 volumes of *2-propanol* and 10 volumes of *0.1 M hydrochloric acid* shows absorption maxima at about 264 nm, 272 nm and 280 nm; ratio of the absorbance at the maximum at about 272 nm to that at the maximum at about 280 nm, 1.18 to 1.22.

C. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

D. It gives the reactions of nitrates (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in the mobile phase and dilute to 10.0 ml with the mobile phase.

Reference solution (a). A solution containing 0.0025 per cent w/v each of *miconazole nitrate IPRS* and *econazole nitrate IPRS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: dissolve 6.0 g of *ammonium acetate* in a mixture of 300 volumes of *acetonitrile*, 320 volumes of *methanol* and 380 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to econazole and miconazole is not less than 10.0.

Inject reference solution (b) and the test solution. Run the chromatogram 1.2 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) and the peak due to nitrate ion.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Dissolve 0.4 g in 50 ml of *anhydrous glacial acetic acid*, with slight heating if necessary. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04791 g of $C_{18}H_{14}Cl_4N_2O,HNO_3$.

Storage. Store protected from light and moisture.

Miconazole Cream

Miconazole Nitrate Cream

Miconazole Cream contains Miconazole Nitrate in a suitable cream base.

Miconazole Cream contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O, HNO_3$.

Usual strength. 2 per cent w/w.

Identification

A. Mix a quantity containing 40 mg of Miconazole Nitrate with 20 ml of a mixture of 4 volumes of *methanol* and 1 volume of *1 M sulphuric acid* and shake with two quantities, each of 50 ml, of *hexane*, discarding the organic layers. Make the aqueous phase alkaline with *2 M ammonia* and extract with two quantities, each of 40 ml of *chloroform*. Combine the *chloroform* extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and dilute the filtrate to 100 ml with *chloroform*. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 90 volumes of *methanol* and 10 volumes of *0.1 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 264 nm, 272 nm and 280 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *tetrahydrofuran*.

Test solution. Shake a quantity of the cream containing 50 mg of Miconazole Nitrate with 30 ml of the solvent mixture for 30 minutes, dilute to 50.0 ml with the solvent mixture and filter through a glass microfiber filter (Such as Whatman GF/C).

Reference solution (a). A 0.1 per cent w/v solution of *miconazole nitrate IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.0025 per cent w/v each of *miconazole nitrate IPRS* and *econazole nitrate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (3 µm) (Such as Hypersil 3 ODS),
- mobile phase: a solution containing 0.6 per cent w/v of *ammonium acetate* in a mixture of 30 volumes of *acetonitrile*, 32 volumes of *methanol* and 38 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume: 10 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to miconazole and econazole is not less than 10.0.

Inject reference solution (b) and the test solution. Run the chromatogram 1.2 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent) and the sum of areas of all the secondary peaks is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore the peak due to nitrate ion.

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{14}Cl_4N_2O, HNO_3$ in the cream.

Storage. Store protected from light at a temperature not exceeding 30°. If it is packed in aluminium tubes the inner surfaces of the tubes should be coated with a suitable lacquer.

Miconazole Pessaries

Miconazole Nitrate Pessaries; Miconazole Nitrate Vaginal Tablets; Miconazole Tablets

Miconazole Pessaries contain Miconazole Nitrate in a suitable base.

Miconazole Pessaries contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of miconazole nitrate, $C_{18}H_{14}Cl_4N_2O, HNO_3$.

Usual strength. 150 mg.

Identification

A. Mix a quantity of the crushed pessaries containing 40 mg of Miconazole Nitrate with 20 ml of a mixture of 4 volumes of *methanol* and 1 volume of *1 M sulphuric acid* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*, discarding the organic layers. Make the aqueous phase alkaline with *2 M ammonia* and extract with two quantities, each of 40 ml, of *chloroform*. Combine the chloroform extracts, shake with 5 g of *anhydrous sodium sulphate*, filter and dilute the filtrate to 100 ml with *chloroform*. Evaporate 50 ml to dryness and dissolve the residue in 50 ml of a mixture of 90 volumes of *methanol* and 10 volumes of *0.1 M hydrochloric acid*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 264 nm, 272 nm and 280 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to miconazole nitrate in the chromatogram obtained with reference solution (a).

Tests

Other tests. Comply with the tests stated under Pessaries.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Weigh a quantity of the crushed pessaries containing about 40 mg of Miconazole Nitrate, mix with 20 ml of a mixture of 4 volumes of *methanol* and 1 volume of *0.5 M sulphuric acid* and shake with two quantities, each of 50 ml, of *carbon tetrachloride*. Wash each organic layer in turn with the same 10-ml quantity of a mixture of 4 volumes of *methanol* and 1 volume of *0.5 M sulphuric acid*. Combine the aqueous phase and the washings, make alkaline with *2 M ammonia* and extract with two quantities, each of 50 ml, of *chloroform*. To the combined extracts add 10.0 ml of a 0.3 per cent w/v solution of *cetyl palmitate* (internal standard) in *chloroform* and 5 g of *anhydrous sodium sulphate*, shake, filter, evaporate the filtrate to a low volume and add sufficient *chloroform* to produce 10.0 ml.

Reference solution (a). Weigh 40 mg of *miconazole nitrate IPRS* and mix with 10.0 ml of a 0.3 per cent w/v solution of the internal standard in *chloroform* and 0.2 ml of *strong ammonia solution*, add 1 g of *anhydrous sodium sulphate*, shake again and filter.

Reference solution (b). Prepare the solution in the same manner as reference solution (a) but omitting the addition of the internal standard solution.

Chromatographic system

- a glass column 1.5 m × 2 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) (Such as 5 per cent OV-101),
- temperature:
  column 270°,
  inlet port and detector at 300°,
- flow rate: 30 ml per minute, using nitrogen as the carrier gas.

Inject 1 µl of the test solution, reference solution (a) and reference solution (b).

Calculate the content of $C_{18}H_{14}Cl_4N_2O, HNO_3$ in the pessaries.

Storage. Store protected from light and moisture.

Microcrystalline Cellulose

Microcrystalline Cellulose is purified, partially depolymerised cellulose prepared from alpha cellulose.

Microcrystalline Cellulose contains not less than 97.0 per cent and not more than 102.0 per cent of cellulose, calculated on the dried basis.

Category. Pharmaceutical aid (suspending agent; tablet and capsule adjuvant).

Description. A fine or granular, white or almost white powder.

Identification

A. To about 1 mg add 1 ml of *phosphoric acid*, heat on a water-bath for 30 minutes, add 4 ml of a 0.2 per cent w/v solution of *catechol* in *phosphoric acid* and heat for further 30 minutes; a red colour is produced.

B. To 50 mg add 2 ml of *iodine solution*, allow to stand for 5 minutes and remove the excess reagent with the aid of a filter paper and add 1 or 2 drops of *sulphuric acid (66 per cent v/v)*; a blue-purple colour is produced.

C. Mix 30 g with 270 ml of *water*, mix in a blender at 18,000 rpm for 5 minutes, transfer 100 ml of the mixture to a 100-ml graduated cylinder and allow to stand for 3 hours. A white, opaque, bubble-free dispersion is obtained that does not produce a supernatant liquid.

Tests

pH (2.4.24). 5.0 to 7.5, determined on the supernatant liquid obtained by shaking 2.0 g with 100 ml of *carbon dioxide-free water* for 5 minutes and centrifuging.

Starch and dextrins. Mix 0.1 g with 5 ml of *water* by vigorous shaking and add 2 to 3 drops of *iodine solution*; no blue or brownish-red colour is produced.

Organic impurities. Place 10 mg on a watch-glass and add 0.05 ml of a freshly prepared solution of 0.1 g of *phloroglucinol* in 5 ml of *hydrochloric acid*; no red colour is produced.

Water-soluble substances. Shake 5.0 g with about 80 ml of *water* for 10 minutes, filter through a filter paper (Whatman No 42 or equivalent) into a tared beaker and evaporate the filtrate to dryness and dry the residue at 105° for 1 hour. The residue weighs not more than 10 mg (0.2 per cent).

Arsenic (2.3.10). Mix 5.0 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in a mixture of 15 ml of *hydrochloric acid* containing 0.15 ml of *bromine solution* and 45 ml of *water*. Add 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Weigh 0.125 g and transfer to a 300-ml conical flask with the aid of about 25 ml of *water*. Add 50.0 ml of *0.083 M potassium dichromate*, mix, carefully add 100 ml of *sulphuric acid* and heat to boiling. Remove from heat, allow to stand at room temperature for 15 minutes, cool and transfer to a 250-ml volumetric flask. Dilute with *water* almost to volume, cool to 25°, dilute with *water* to volume and mix. Titrate 50.0 ml of the resulting solution with *0.1 M ferrous ammonium sulphate* using 2 to 3 drops of *ferroin sulphate solution* as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of ferrous ammonium sulphate required.

1 ml of *0.1 M ferrous ammonium sulphate* is equivalent to 0.000675 g of cellulose.

Storage. Store protected from light and moisture.

Microcrystalline Cellulose and Carboxymethylcellulose Sodium

Microcrystalline Cellulose and Carboxymethylcellulose Sodium is a colloid-forming, attrited mixture of Microcrystalline Cellulose and Carboxymethylcellulose Sodium.

Microcrystalline Cellulose and Carboxymethylcellulose Sodium contains not less than 75.0 per cent and not more than 125.0 per cent of carboxymethylcellulose sodium, calculated on the dried basis. The viscosity of its aqueous dispersion of per cent by weight stated on the label is not less than 60.0 per cent and not more than 140.0 per cent of that stated on the label in centipoises.

Identification

Mix 6 g with 300 ml of *water*, mix in a blender at 18,000 rpm for 5 minutes, transfer 100 ml of the mixture to a 100-ml graduated cylinder and allow to stand for 3 hours. A white, opaque, dispersion is produced which does not settle on standing.

A. Add few drops of the dispersion obtained to a 10 per cent w/v solution of *aluminum chloride*, each drop forms a white, opaque globule which does not disperse on standing.

B. Add 3 ml of *iodine solution* to the dispersion obtained; no blue or purplish blue color is produced

Tests

pH (2.4.24). 6.0 to 8.0, determined in the dispersion prepared in the test for Viscosity.

Viscosity (2.4.28). Determine the amounts of Microcrystalline Cellulose and Carboxymethylcellulose Sodium needed to prepare 600 g of a suitable dispersion, calculated on the dried basis. Transfer a weighed amount of *water* to a 1000-ml blender bowl. Begin stirring with an 18,000 rpm blender at a reduced speed obtained by adjusting the voltage to 30 volts by means of a suitable transformer, and immediately add the weighed portion of Microcrystalline Cellulose and Carboxymethyl cellulose Sodium, taking care to avoid contacting the sides of the bowl with the powder. Continue stirring at this speed for 15 seconds following the addition of the powder, then increase the transformer setting to 115 volts, and mix for 2 minutes, accurately timed, at 18,000 rpm. Stop the blender, and lower the appropriate spindle of a suitable rotational viscometer into the dispersion. Thirty seconds after cessation of mixing, start the viscosimeter, and determine the viscosity using the appropriate spindle to obtain a scale reading between 10 per cent and 90 per cent of full-scale at a speed of 20 rpm. Determine the scale reading after 30 seconds of rotation, and calculate the viscosity, in centipoises, by multiplying the scale reading by the constant for the spindle used at 20 rpm.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 5.0 per cent.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 2 g of Microcrystalline Cellulose and Carboxymethylcellulose Sodium and transfer to a 250-ml of a glass-stoppered conical flask, add 75 ml of *glacial acetic acid*, attach a condenser, and reflux for 2 hours, cool, transfer the mixture to a 250-ml beaker with the aid of small volumes of

glacial acetic acid. Titrate with *0.1 M perchloric acid* in *dioxane solution*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0296 g of carboxymethylcellulose sodium.

Storage. Store protected from light and moisture.

Labelling. Label it to indicate the percentage content of carboxymethylcellulose sodium and the viscosity of the dispersion in water of the designated weight percentage composition.

Microcrystalline Wax

Petroleum Wax (microcrystalline); Amorphous Wax

Microcrystalline Wax is a mixture of straight-chain, branched-chain and cyclic hydrocarbons, obtained by solvent fractionation of the still bottom fraction of petroleum by suitable dewaxing or de-oiling means.

Category. Pharmaceutical aid (stiffening and coating agent; ointment base).

Description. A white or cream-coloured waxy solid.

Tests

Acidity or alkalinity. Introduce 35.0 g into a 250-ml separating funnel, add 100 ml of boiling *water* and shake vigorously for 5 minutes. Draw off the separated water into a beaker, wash further with two quantities, each of 50 ml, of boiling *water* and add the washings to the liquid in the beaker. To the pooled washings add 0.05 ml of *phenolphthalein solution* and boil; the solution does not acquire a pink colour. Cool, add 0.1 ml of *methyl orange solution*; no red or pink colour is produced.

Solidifying point (2.4.10). 54° to 102°. Follow the method with the following modifications. Place in the inner test-tube sufficient of the melted substance to fill the tube to a depth of about 50 mm. Stir the substance gently and steadily, without scraping the side of the tube, while the tube and its contents are allowed to cool. The temperature at which the level of the mercury in the thermometer remains stationary for a short time is taken as the solidifying point.

Colour. Melt about 10.0 g on a water-bath and pour 5 ml of the liquid into a clear-glass (15 cm × 16 mm) bacteriological test-tube; the warm, melted liquid is not more intensely coloured than a solution prepared by mixing 3.8 ml of FCS and 1.2 ml of CCS (2.4.1), in a similar tube, the comparison being made in reflected light against a white background, the tubes being held directly against the background at such an angle that there is no fluorescence.

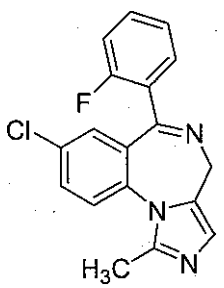
Organic acids. To 20.0 g add 100 ml of a 50 per cent v/v solution of *ethanol (95 per cent)* neutralised to *phenolphthalein solution* and titrate rapidly with *0.1 M sodium hydroxide* with vigorous agitation, to a sharp pink end-point. Not more than 0.4 ml of *0.1 M sodium hydroxide* is required.

Fats, fixed oils and rosin. Digest 10.0 g with 10 ml of *5 M sodium hydroxide* at 100° for 30 minutes. Separate the water layer and acidify with *sulphuric acid*; no oily or solid matter separates.

Ash (2.3.19). Not more than 0.1 per cent, determined on 2.0 g. It volatilises without emitting an acrid odour.

Storage. Store protected from light and moisture.

Midazolam



$C_{18}H_{13}ClFN_3$ Mol. Wt. 325.8

Midazolam is 8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine.

Midazolam contains not less than 98.5 per cent and not more than 101.5 per cent calculated on the dried basis.

Category. Sedative; Anxiolytic.

Description. A white or yellowish, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *midazolam IPRS* or with the reference spectrum of midazolam.

B. In the test for Impurity C, the principal spot in the chromatogram obtained with test solution (a) corresponds to that in the chromatogram obtained with reference solution (b).

C. Mix 90 mg with 0.3 g of *anhydrous sodium carbonate* and ignite in a crucible until an almost white residue is obtained. Allow to cool and dissolve the residue in 5 ml of *dilute nitric*

acid, filter. Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml of *alizarin S solution* and 0.1 ml of *zirconyl nitrate solution*. Mix, allow to stand for 5 minutes and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank solution is red.

D. To 1 ml of the filtrate obtained in identification test C add 1 ml of *water*. The solution gives reaction (A) of chlorides (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *0.1 M hydrochloric acid* is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *methanol* and dilute to 50.0 ml with *methanol*.

Reference solution. A 0.0001 per cent w/v solution of *midazolam IPRS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 44 volumes of a solution containing 0.77 per cent w/v of *ammonium acetate* and 1.0 per cent v/v of *tetrabutylammonium hydroxide solution* (40 per cent w/v), adjusted to pH 5.3 with *glacial acetic acid* and 56 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Midazolam impurity I[1]	0.25	—
Midazolam impurity J[2] (2 peaks)	0.3	—
Midazolam impurity D[3]	0.4	—
Midazolam impurity E[4]	0.5	2.0
Midazolam impurity F[5]	0.7	—
Midazolam impurity A[6]	0.9	2.0
Midazolam (Retention time: about 17 minutes)	1.0	—
Midazolam impurity G[7]	1.2	—
Midazolam impurity H[8]	1.9	1.7
Midazolam impurity B[9]	2.2	—

[1](3a*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-3a,4-dihydro-3*H*-imidazo[1,5-*a*][1,4]benzodiazepine,

[2]8-chloro-6-(2-fluorophenyl)-1-methyl-3a,4,5,6-tetrahydro-3*H*-imidazo[1,5-*a*][1,4]benzodiazepine,

[3]8-chloro-6-(2-fluorophenyl)-1-methyl- 4*H*-imidazo[1,5-*a*][1,4] benzodiazepine 5-oxide,

[4][(2*RS*)-7-chloro-5-(2-fluorophenyl)2,3-dihydro-1*H*-1,4-benzodiazepine-2-yl]methanamine,

[5]7-chloro-5-(2-fluoropheny)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (1-des[(diethylamino)ethyl] flurazepam),

[6](6*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-5,6-dihydro-4*H*-imidazo [1,5-*a*][1,4]benzodiazepine,

[7]desfluoromidazolam,

[8]6-chloro-4-(2-fluorophenyl)-2-methylquinazoline,

[9](6*RS*)-8-chloro-6-(2-fluorophenyl)-1-methyl-6*H*-imidazo[1,5-*a*][1,4] benzodiazepine.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. Run the chromatogram 3 times the retention time of the principal peak. The area of any peak due to midazolam impurity B is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of any peak due to midazolam impurities A and G is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent). Ignore any peak with the area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Impurity C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 2 volumes of *glacial acetic acid*, 15 volumes of *water*, 20 volumes of *methanol* and 80 volumes of *ethyl acetate*.

Test solution (a). Dissolve 0.2 g of the substance under examination in *ethanol (95 per cent)* and dilute to 5 ml with *methanol*.

Test solution (b). Dilute 1 ml of test solution (a) to 50 ml with *ethanol (95 per cent)*.

Reference solution (a). Dissolve the contents of a vial of *midazolam impurity C (8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylic acid) IPRS* in 2.0 ml of *methanol*.

Reference solution (b). A 0.08 per cent w/v solution of *midazolam IPRS* in *ethanol (95 per cent)*.

Reference solution (c). Dissolve 40 mg of the substance under examination in 1 ml of reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a) due to midazolam impurity C is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.1 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows 2 clearly separated spots.

Sulphated ash (2.3.18). Not more than 0.1 per cent in a platinum crucible.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Dissolve 0.12 g in 30 ml of *anhydrous acetic acid* and add 20 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid* to the 2nd point of inflexion, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M perchloric acid* is equivalent to 0.01629 g of $C_{18}H_{13}ClFN_3$.

Storage. Store protected from light.

Midazolam Injection

Midazolam Injection is a sterile solution of Midazolam in Water for Injections containing hydrochloric acid.

Midazolam Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of midazolam, $C_{18}H_{13}ClFN_3$.

Usual strengths. 1 mg per ml; 5 mg per ml.

Identification

To a volume of the injection containing 20 mg of Midazolam add sufficient *5 M ammonia* to make the solution just alkaline, extract with two 10- ml quantities of *dichloromethane*, dry the combined extracts over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *midazolam IPRS* or with the reference spectrum of midazolam.

Tests

pH (2.4.24). 2.9 to 3.7.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Keep the solutions protected from light atleast for 2 hours before testing.

Test solution. Dilute a volume of the injection containing 10 mg of Midazolam to 20.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.001 per cent w/v each of *N-desalkylflurazepam IPRS* and *midazolam IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 28 volumes of a solution containing equal volumes of *0.1 M orthophosphoric acid* and *0.03 M triethylamine*, adjusted to pH 3.5 with *0.1 M sodium hydroxide* and 72 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to midazolam and *N*-desalkylflurazepam is not less than 3.0.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Bacterial endotoxins (2.2.3). Not more than 8.33 Endotoxin Units per mg of midazolam.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Keep the solutions protected from light atleast for 2 hours before testing.

Test solution. Dilute a volume of the injection containing 10 mg of Midazolam to 100 ml with the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *midazolam IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.001 per cent w/v each of *N-desalkylflurazepam IPRS* and *midazolam IPRS* in the mobile phase.

Use chromatographic system as described in the Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to midazolam and *N*-desalkylflurazepam is not less than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{13}ClFN_3$ in the injection.

Storage. Store protected from light.

Midazolam Oral Solution

Midazolam Oral Solution is a solution of Midazolam in a suitable flavoured vehicle.

Midazolam Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of midazolam, $C_{18}H_{13}ClFN_3$.

Identification

A. To a volume of the oral solution containing 20 mg of Midazolam add sufficient *5 M ammonia* to make the solution just alkaline, extract with two 10-ml quantities of *dichloromethane*, dry the combined extracts over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *midazolam IPRS* or with the reference spectrum of midazolam.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Protect the solutions from light for at least 2 hours before testing.

Test solution. Dilute a volume of the oral solution containing 10 mg of Midazolam to 20.0 ml with the mobile phase, filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.001 per cent w/v each of *N-desalkylflurazepam IPRS* and *midazolam IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica),
- column temperature: 40°,
- mobile phase: a mixture of 28 volumes of a solution prepared by mixing equal volumes of *0.1 M orthophosphoric acid* and *0.03 M triethylamine*, adjusted to pH 3.5 with *0.1 M sodium hydroxide* and 72 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Inject reference solution (b).The test is not valid unless the resolution between the peaks due to midazolam and *N*-desalkylflurazepam is not less than 3.0.

Inject reference solution (a) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent).

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

NOTE— Protect the solutions from light for at least 2 hours before testing.

Test solution. Dilute a volume of the oral solution containing 10 mg of Midazolam to 100.0 ml with the mobile phase, filter.

Reference solution (a). A 0.01 per cent w/v solution of *midazolam IPRS* in the mobile phase.

Reference solution (b). A solution containing 0.001 per cent w/v each of *N-desalkylflurazepam IPRS* and *midazolam IPRS* in the mobile phase.

Use chromatographic system as described in the Related substances.

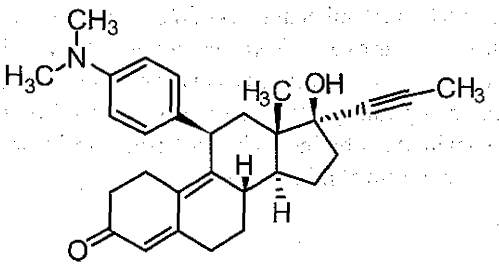
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to midazolam and *N*-desalkylflurazepam is not less than 3.0.

Inject reference solution (a) and the test solution.

Determine the weight per ml (2.4.29) and calculate the content of $C_{18}H_{13}ClFN_3$ in the oral solution.

Storage. Store protected from light.

Mifepristone



$C_{29}H_{35}NO_2$ Mol Wt. 429.6

Mifepristone is 17β-Hydroxy-11β-(4-dimethylamino)phenyl-17-(1-propynyl)-4,9-estradien-3-one.

Mifepristone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{29}H_{35}NO_2$, calculated on the dried basis.

Category. Abortifacient.

Description. A slightly yellow crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mifepristone IPRS* or with the reference spectrum of mifepristone.

Tests

Light absorption. Not more than 1.25, determined in a 1.0 per cent w/v solution in *methanol* at 420 nm (2.4.7).

Specific optical rotation (2.4.22). +124° to +135°, determined in a 0.5 per cent w/v solution in *dichloromethane* at 20°.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *methanol.*

Test solution. Dissolve 25 mg of the substance under examination in 5 ml of *methanol* and dilute to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4 mm packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature: 40°,
- sample temperature: 5°,
- mobile phase: A. dilute 0.75 ml of *formic acid* to 1000 ml with *water*, adjusted to pH 5.0 with dilute *ammonium hydroxide solution*,
  B. a mixture of equal volumes of *methanol* and *acetonitrile*,
- a gradient programme using the condition given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume: 10 µl

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	40	60
30	34	66
50	10	90
60	10	90
65	40	60
70	40	60

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of the area of all the secondary peaks is not more than 1.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 1.0 g complies with the limit for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh 0.3 g and dissolve in 50 ml of *glacial acetic acid.* Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (2.4.25). Carry out blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04296 g of $C_{29}H_{35}NO_2$.

Mifepristone Tablets

Mifepristone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mifepristone, $C_{29}H_{35}NO_2$.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows absorption maxima at 260 nm and 303 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 1.0 per cent w/v solution of *sodium lauryl sulphate* in *water*,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the dissolution medium, if necessary, at the maximum at about 302 nm (2.4.7). Calculate the content of $C_{29}H_{35}NO_2$ in the medium from the absorbance obtained from a solution of known concentration of *mifepristone IPRS*, prepared by dissolving in suitable quantity in *methanol* and diluted with the dissolution medium in such a manner to get similar concentration of the test solution.

Q. Not less than 70 per cent of the stated amount of $C_{29}H_{35}NO_2$.

Related substances. Determine by liquid chromatography (2.4.14), as described in the assay with the following modifications.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.0 per cent and the sum of areas of all the secondary peaks is not more than 2.0 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 50 mg of Mifepristone in 20 ml of the mobile phase with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with the mobile phase, centrifuge. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *mifepristone IPRS* in the mobile phase.

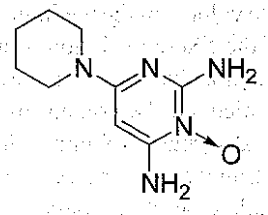
Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 21 volumes of *methanol*, 14 volumes of *acetonitrile*, 15 volumes of *water* and 0.1 volume of *triethylamine*, adjusted to pH 4.5 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor for the principal peak is not more than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{29}H_{35}NO_2$ in the tablets.

Minoxidil



$C_9H_{15}N_5O$ Mol. Wt. 209.3

Minoxidil is 2,4-diamino-6-piperidinopyrimidine 3-oxide.

Minoxidil contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_{15}N_5O$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *minoxidil IPRS* or with the reference spectrum of minoxidil.

B. Dissolve about 20mg in *0.1 M hydrochloric acid* and dilute to 100 ml with the same solvent (solution A). Dilute 2 ml of solution A to 100 ml with *0.1 M hydrochloric acid* (solution B) and dilute 2 ml of solution A to 100 ml with *0.1 M sodium hydroxide* (solution C).

Measure the light absorption of solutions B and C in the range 220 nm to 360 nm (2.4.7). Solution B shows absorption maxima at about 230 nm and 281 nm; absorbance at about 230 nm, 0.406 to 0.448 and at about 281 nm, 0.424 to 0.468. Solution C shows absorption maxima at about 230 nm, 262 nm and 288 nm; absorbance at about 230 nm, 0.610 to 0.674, at about 262 nm, 0.194 to 0.214 and at about 288 nm, 0.222 to 0.242.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

Reference solution. A 0.1 per cent w/v solution of *minoxidil IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve about 10 mg in 1 ml of *methanol*, add 0.1 ml of *cupric sulphate solution*; a green colour develops. The solution becomes greenish-yellow on addition of 0.1ml of *2 M hydrochloric acid*.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *deoxyminoxidil IPRS* in the mobile phase and dilute to 20 ml with the mobile phase. To 2 ml of the solution add 2 ml of the test solution and dilute to 10 ml with the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 3 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: dissolve 3.0 g of *dioctyl sodium sulphosuccinate* in a mixture of 10 ml of *glacial acetic acid*, 300 ml of *water* and 700 ml of *methanol* and adjusted to pH 3.0 with *perchloric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to minoxidil and deoxyminoxidil is not less than 2.0.

Inject reference solution (a), (b) and the test solution. Continue the chromatography for twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of any secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak with an area less than 0.1 times of that of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Heavy metals (2.3.13). Dissolve 1.0 g in 25 ml of *methanol*, the solution complies with the limit test for heavy metals, Method C (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh 0.15 g, dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02093 g of $C_9H_{15}N_5O$.

Storage. Store protected from light and moisture.

Minoxidil Tablets

Minoxidil Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of minoxidil, $C_9H_{15}N_5O$.

Usual strengths. 2.5 mg; 5 mg; 10 mg.

Identification

A. Transfer a portion of the finely powdered tablets containing about 10 mg of Minoxidil to a separator, add 25 ml of *water*, and extract with three quantities, each of 15 ml, of *chloroform*. Combine the chloroform extracts and evaporate with the aid of stream of nitrogen. Wash the inside of the container with about 5 ml of *ethanol (95 per cent)*, add 0.3 g of *potassium bromide IR* and evaporate under vacuum at 50° until dry. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *minoxidil IPRS* or with the reference spectrum of minoxidil.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 10 mg of Minoxidil with 10 ml of *methanol*, centrifuge and use the supernatant liquid.

Reference solution. A 0.1 per cent w/v solution of *minoxidil IPRS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 7.2*,
Speed and time. 75 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 231 nm (2.4.7), for tablets containing up to 10 mg Minoxidil; for tablets containing more than 10 mg of Minoxidil the wavelength used is about 287 nm. Similarly measure the absorbance of a solution of known concentration of *minoxidil IPRS*. Calculate the content of $C_9H_{15}N_5O$.

Q. Not less than 75 per cent of the stated amount of $C_9H_{15}N_5O$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 5 mg of Minoxidil with 20 ml of a solution of *medroxyprogesterone acetate* (internal standard) in the mobile phase having a concentration of about 0.2 mg per ml (solution A) for 5 minutes and centrifuge.

Reference solution. Dissolve a weighed quantity of *minoxidil IPRS* in solution A to obtain a solution having a known concentration of about 0.25 mg per ml.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution prepared by adding 3.0 g of *docusate sodium* per litre to a mixture of 700 volumes of *methanol*, 300 volumes of *water* and 10 volumes of *glacial acetic acid*, adjusting to pH 3.0 with *perchloric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

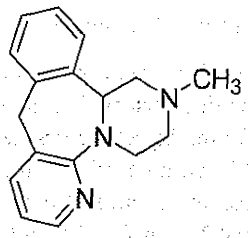
Chromatograph not less than 4 replicate injections of reference solution and record the peak response as mentioned below. The relative standard deviation is not more than 2.0 per cent and the resolution between the internal standard and minoxidil is not less than 2.0.

Inject the reference solution and the test solution. The relative retention times are about 0.8 for the internal standard and 1.0 for minoxidil.

Calculated the content of minoxidil, $C_9H_{15}N_5O$ in the tablets.

Storage. Store protected from light.

Mirtazapine



$C_{17}H_{19}N_3$ Mol. Wt. 265.4

Category. Antidepressant.

Mirtazapine is (14b*RS*)-2-methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*] [2]benzazepine.

Mirtazapine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{19}N_3$, calculated on the anhydrous basis.

Description. A white or almost white powder, slightly hygroscopic to hygroscopic in nature. It shows polymorphism (2.5.11).

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mirtazapine IPRS* or with the reference spectrum of mirtazapine.

Tests

Optical rotation (2.4.22). – 0.10° to + 0.10°, determined in a 1.0 per cent w/v solution in *ethanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Dissolve 30 mg of the substance under examination in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 7.5 volumes of *tetrahydrofuran*, 12.5 volumes of *methanol*, 15 volumes of *acetonitrile* and 65 volumes of buffer solution prepared by dissolving 18.0 g of *tetramethylammonium hydroxide* in 950 ml of *water*, adjusted to pH 7.4 with *orthophosphoric acid*, then dilute to 1000 ml with *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Mirtazapine impurity A[1]	0.2	1.3
Mirtazapine impurity B[2]	0.3	1.3
Mirtazapine impurity C[3]	0.35	—
Mirtazapine impurity D[4]	0.4	—
Mirtazapine (retention time: about 25 minutes)	1.0	—
Mirtazapine impurity E[5]	1.3	—
Mirtazapine impurity F[6]	1.35	0.2

[1](14b*RS*)-2-methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido [2,3-*c*] [2]benzazepine 2-oxide,

[2][2-[(2*RS*)-4-methyl-2-phenylpiperazin-1-yl]pyridin-3-yl]methanol,

[3](14b*RS*)-2-methyl-3,4,10,14b-tetrahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepin-1(2*H*)-one,

[4](14b*RS*)-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine,

[5] (2*RS*)-4-methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine,

[6](14b*RS*)-2-methyl-1,3,4,14b-tetrahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepin-10(2*H*)-one.

Inject the reference solution. The test is not valid unless the tailing factor for the principal peak is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates.

Inject the reference solution and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 3.5 per cent, determined on 1.0 g.

Assay. Dissolve 0.1 g in 35 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M perchloric acid* is equivalent to 0.01327 g of $C_{17}H_{19}N_3$.

Storage. Store protected from moisture.

Mirtazapine Tablets

Mirtazapine Tablets contains not less than 95.0 per cent and not more than 105.0 per cent of $C_{17}H_{19}N_3$.

Usual strengths. 15 mg; 30 mg; 45 mg.

Identification

Mix a quantity of the powdered tablets containing 50 mg of Mirtazapine with 12.5 ml of *water*, add 12.5 ml of *n-hexane*, allow to separate, retain and filter the hexane layer and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mirtazapine IPRS* or with the reference spectrum of mirtazapine.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *mirtazapine IPRS* in the dissolution medium to obtain a solution of known concentration similar to the expected concentration of the test solution.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with base deactivated octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C18),
- column temperature: 40°,
- mobile phase: a mixture of 80 volumes of a solution containing 2 volumes of *triethylamine* and 1000 volumes of a 0.68 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 3.0 with *dilute orthophosphoric acid* and 20 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 292 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_3$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{17}H_{19}N_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Disperse a quantity of the powdered tablets containing 30 mg of Mirtazapine with 10 ml of the solvent mixture with the aid of ultrasound, dilute to 20.0 ml with the solvent mixture and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of *tetrahydrofuran*, 125 volumes of *methanol*, 150 volumes of *acetonitrile* and 650 volumes of a solution of 1.8 per cent w/v of *tetramethylammonium hydroxide*, adjusted to pH 7.4 with *orthophosphoric acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Mirtazapine impurity A[1]	0.2	1.3
Mirtazapine impurity B[2]	0.3	1.3
Mirtazapine impurity C[3]	0.35	---
Mirtazapine impurity D[4]	0.4	---
Mirtazapine (retention time: about 16 minutes)	1.0	---
Mirtazapine impurity E[5]	1.3	---
Mirtazapine impurity F[6]	1.35	0.2

[1](14b*RS*)-2-methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*] [2]benzazepine 2-oxide,

[2][2-[(2*RS*)-4-methyl-2-phenylpiperazin-1-yl]pyridin-3-yl]methanol,

[3](14b*RS*)-2-methyl-3,4,10,14b-tetrahydropyrazino[2,1-*a*]pyrido[2,3-*c*] [2]benzazepin-1(2*H*)-one,

[4](14b*RS*)-1,2,3,4,10,14b-hexahydropyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine,

[5] (2*RS*)-4-methyl-1-(3-methylpyridin-2-yl)-2-phenylpiperazine,

[6](14b*RS*)-2-methyl-1,3,4,14b-tetrahydropyrazino[2,1-*a*]pyrido[2,3-*c*] [2]benzazepin-10(2*H*)-one.

Inject reference solution (b). The test is not valid unless the signal-to-noise ratio of the principal peak is not less than 10.

Inject reference solution (a), (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 90 mg of Mirtazapine with 50 ml of the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution. A 0.009 per cent w/v solution of *mirtazapine IPRS* in the solvent mixture.

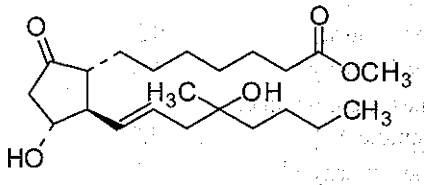
Use chromatographic system as described in the Related substances using a detection wavelength of 290 nm.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{17}H_{19}N_3$ in the tables.

Misoprostol



$C_{22}H_{38}O_5$ Mol. Wt. 382.5

Misoprostol is (*RS*)-methyl (13*E*)-11,16-dihydroxy-16-methyl-9-oxoprost-13-enoate.

Misoprostol contains not less than 96.5 per cent and not more than 102.0 per cent of $C_{22}H_{38}O_5$, calculated on the anhydrous basis.

Category. Abortifacient; gastric cytoprotector.

Description. A clear, colourless or yellowish oily liquid.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *misoprostol IPRS* or with the reference spectrum of misoprostol.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 5.0 ml of *acetonitrile*.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile* .

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with *acetonitrile*.

Reference solution (c). A 0.0025 per cent w/v solution of *misoprostol impurity A IPRS* in reference solution (a).

Reference solution (d). A 0.2 per cent w/v solution of *misoprostol IPRS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 45 volumes of *acetonitrile*, 55 volumes of *water* and 0.05 volume of 2.45 per cent w/v solution of *orthophosphoric acid*,
- flow rate: 0.75 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume: 10 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to misoprostol impurity A and misoprostol is not less than 1.9. The relative retention time with reference to misoprostol for 8-epimisoprostol (misoprostol impurity A) is about 0.9, for 11-epi misoprostol (misoprostol impurity E) is about 0.9, for 12-epimisoprostol (misoprostol impurity B) (1st peak) is about 0.9 and for misoprostol impurity B (2nd peak) is about 0.95.

Inject reference solution (a), (b) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. The sum of areas of the peaks due to misoprostol impurity A, B and E is not more than 1.3 times the area of the principal

peak in the chromatogram obtained with reference solution (a) (1.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Diastereoisomers. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 20 mg of the substance under examination in 1.0 ml of the mobile phase.

Reference solution. Dilute 0.1 ml of the test solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 5 volumes of *2-propanol*, 95 volumes of *heptane* and 0.01 volume of *glacial acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the resolution between the 1st peak and the 2nd peak of misoprostol is not less than 2.3. The retention time of Misoprostol 1st peak is about 19 minutes and misoprostol 2nd peak about 21 minutes.

Inject the reference solution and the test solution. Run the chromatogram 1.5 times the retention time of the 1st peak of misoprostol. In the chromatogram obtained with the test solution, the area of the 1st peak of misoprostol is 50 per cent to 55 per cent of the sum of the areas of the 2 peaks due to misoprostol.

Water (2.3.43). Not more than 1.0 per cent, determined on 1 g.

Assay. Determine by liquid chromatography (2.4.14), as described in the Related substances.

Inject reference solution (d) and the test solution.

Calculate the content of $C_{22}H_{38}O_5$.

Storage. Store protected from moisture, at a temperature of about – 20°.

Misoprostol Tablets

Misoprostol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of misoprostol, $C_{22}H_{38}O_5$.

Usual strengths. 25 µg; 100 µg; 200 µg; 600 µg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Disintegration (2.5.1). Not more than 15 minutes.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14) as described in the Assay using following modifications.

Test solution. Disperse 1 tablet in the mobile phase and dilute to 25.0 ml with the mobile phase, filter.

Reference solution. Dissolve a weighed quantity of *misoprostol IPRS* in the mobile phase and dilute with the mobile phase to obtain a solution having a known concentration similar to the expected concentration of the test solution.

- injection volume: 50 µl.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.4 mg of Misoprostol in the mobile phase with the aid of ultrasound for 15 minutes, cool and dilute to 10.0 ml with the mobile phase, filter.

Reference solution. A 0.004 per cent w/v solution of *misoprostol IPRS* in the mobile phase.

Chromatographic system

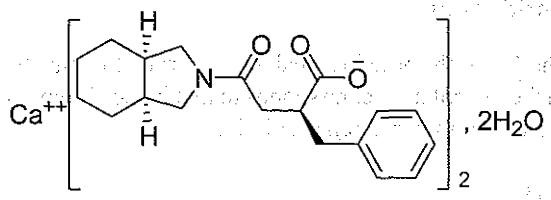
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 30 volumes of buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adjusting to pH 3.0 with *orthophosphoric acid* and 70 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{38}O_5$ in the tablets.

Mitiglinide Calcium Dihydrate



$C_{38}H_{48}CaN_2O_6,2H_2O$ Mol Wt. 704.9

Mitiglinide Calcium Dihydrate is calcium (*S*)-2-benzyl-4-((3a*R*,7a*S*)-hexahydro-1*H*-isoindol-2(3*H*)-yl)-4-oxobutanoate dehydrate.

Mitiglinide Calcium Dihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{38}H_{48}CaN_2O_6$, calculated on the anhydrous basis.

Category. Antidiabetic.

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mitiglinide calcium dihydrate IPRS* or with the reference spectrum of *mitiglinide calcium dihydrate.*

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

***R*- isomer.** Not more than 0.5 per cent .

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with mobile phase.

Reference solution. A 0.1 per cent w/v solution of *mitiglinide calcium dihydrate (racemic mixture) IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, such as Sumichiral OA-3100R (5 µm),
- mobile phase: a mixture of 85 volumes of 0.4 per cent v/v solution of *trifluoro acetic acid* in *hexane* and 15 volumes of *isopropyl alcohol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the isomer and mitiglinide calcium dihydrate is not less than 1.5, column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of R-isomer by area normalisation.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture: 50 volumes of water and 50 volumes of *methanol.*

Test solution. Dissolve 40 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution. A 0.0004 per cent w/v solution of *mitiglinide calcium dihydrate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase:. a mixture of 55 volumes of 0.02 *M potassium dihydrogen phosphate* in *water,* adjusted to pH 4.0 with *orthophosphoric acid,* and 45 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with test solution the area of any secondary peak is not more than 0.5 times the area of principal peak in the chromatogram obtained with reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 7.0 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture: 50 volumes of water and 50 volumes of *methanol.*

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with solvent mixture. Dilute 10.0 ml of the solution to 25.0 ml with the solvent mixture.

Reference solution. A 0.008 per cent w/v solution of *mitiglinide calcium dihydrate IPRS* in the solvent mixture.

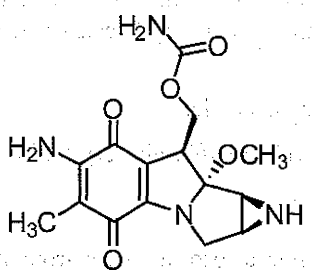
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: a mixture of 40 volumes of 0.02 *M potassium dihydrogen phosphate* in *water*, adjusted to pH 4.0 with *orthophosphoric acid*, and 60 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{38}H_{48}CaN_2O_6$.

Mitomycin



$C_{15}H_{18}N_4O_5$ Mol. Wt. 334.3

Mitomycin is (1*S*,2*S*,8*S*,8a*R*)-[6-Amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazireno[2',3':3,4] pyrrolo[1,2-*a*]-8-indolyl]methylcarbamate.

Mitomycin has a potency of not less than 970 µg per mg calculated on the anhydrous basis.

Category. Anticancer.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mitomycin IPRS* or with the reference spectrum of mitomycin.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.0005 per cent w/v solution in *methanol* shows an absorption maximum at 357 nm as that of solution of *mitomycin IPRS* prepared in the same manner.

Tests

pH (2.4.24). 6.0 to 7.5, determined in a 0.5 per cent w/v solution.

Water (2.3.43). Not more than 2.5 per cent, determined by Method I.

Mitomycin intended for use in the manufacture of parenteral preparations complies with the following additional requirements.

Bacterial endotoxins (2.2.3). Not more than 10.0 Endotoxin Units per mg of Mitomycin.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse 25 mg of the substance under examination in *N,N-dimethylacetamide* and dilute to 50.0 ml with *N,N-dimethylacetamide*.

Reference solution (a). A 0.05 per cent w/v solution of *mitomycin IPRS* in *N,N-dimethylacetamide*.

Reference solution (b). A solution containing 0.05 per cent w/v of *mitomycin IPRS* and 0.75 per cent w/v of *3-ethoxy-4-hydroxybenzaldehyde* in *N,N-dimethylacetamide*.

Chromatographic system
- a stainless steel column 30 cm x 4.0 mm, packed with phenyl groups bonded to porous silica (5 µm),
- mobile phase: dissolve 1.54 g of *ammonium acetate* in 250 ml of *methanol*, add 5.0 ml of *0.83 M acetic acid* and dilute to 1000 ml of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 365 nm,
- injection volume: 10 µl.

The relative retention time with reference to mitomycin for 3-ethoxy-4-hydroxybenzaldehyde is 1.4.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to mitomycin and 3-ethoxy-4-hydroxybenzaldehyde is not less than 1.8, the tailing factor for the principal peak is not more than 1.3 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{15}H_{18}N_4O_5$.

Storage. Store protected from light and moisture.

Mitomycin Injection

Mitomycin Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of mitomycin, $C_{15}H_{18}N_4O_5$.

Usual strength. 5 mg per 10 ml vial.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Identification

Determine by thin-layer chromatography (2.4.17). coating the plate with *silica gel G*.

Mobile phase. A mixture of 4 volumes of *butyl alcohol*, 2 volumes of *glacial acetic acid* and 1 volume of *water*.

Test solution. Dissolve a quantiy of powder of injection containing 10 mg of Mitomycin in *water* and dilute with to 10.0 ml with *water*.

Reference solution. A 0.1 per cent w/v solution of *mitomycin IPRS* in *water*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 1 per cent w/v solution of *ninhydrin* in *ethanol (95 per cent)*, heat at 110° for 15 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.0 to 8.0, where it contain mannitol and 5.5 to 8.5 where it contains hydroxypropyl betadex.

Water (2.3.43). Not more than 5.0 per cent, using Method 1.

Bacterial endotoxins (2.2.3). Not more than 10.0 Endotoxin Units per mg of Mitomycin.

Other tests. Comply with the tests stated under Parenteral Preparations (Powder for Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Add an accurately measured volume of *N,N-dimethylacetamide* to 1 container of mitomycin for injection to obtain a solution containing 0.05 per cent w/v of Mitomycin.

Reference solution (a). A 0.05 per cent w/v solution of *mitomycin IPRS* in *N,N-dimethylacetamide*.

Reference solution (b). A solution containing 0.05 per cent w/v of *mitomycin IPRS* and 0.75 per cent w/v of *3-ethoxy-4-hydroxybenzaldehyde* in *N,N-dimethylacetamide*.

Chromatographic system

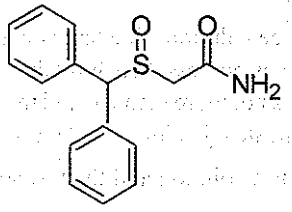
- a stainless steel column 30 cm x 4.0 mm, packed with phenyl groups bonded to porous silica (5 µm),
- mobile phase: dissolve 1.54 g of *ammonium acetate* in 250 ml of *methanol*, add 5.0 ml of *0.83 M acetic acid* and dilute to 1000 ml of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 365 nm,
- injection volume: 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to mitomycin and 3-ethoxy-4-hydroxybenzaldehyde is not less than 1.8, the tailing factor for the principal peak is not more than 1.3 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Calculate the content of $C_{15}H_{18}N_4O_5$ in the injection.

Storage. Store protected from light.

Modafinil



$C_{15}H_{15}NO_2S$ Mol.Wt. 273.4

Modafinil is acetamide, 2-[(diphenylmethyl)sulphenyl]; 2-[(Diphenylmethyl)sulfinyl]-acetamide.

Modafinil contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{15}H_{15}NO_2S$, calculated on the anhydrous basis.

Category. Psychoanaleptic.

Description. A white to off-white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *modafinil IPRS* or with the reference spectrum of modafinil.

Tests

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Name	Relative retention time	Correction factor
Modafinil	1.0	—
Salicylic acid[1]	1.1	—
Modafinil acid[2]	1.4	—
Modafinil sulphone[3]	1.7	1.1
Modafinil ester[4]	3.0	—

[1]salicylic acid is used for calculating resolution and is not a potential impurity,

[2]2-[(diphenylmethyl)sulphenyl]acetic acid,

[3]2-[(diphenylmethyl)sulphonyl]acetamide,

[4]2-[(diphenylmethyl)sulphenyl]acetic acid methyl ester.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to modafinil and salicylic acid is not less than 1.3 and the tailing factor for modafinil peak is not more than 1.5.

Inject the test solution. The area of any peak due to modafinil acid, modafinil sulphone and modafinil ester is not more than 0.5 per cent each, the area of any other secondary peak is not more than 0.05 per cent and the sum of areas of all secondary peaks is not more than 1.0 per cent, calculated by area normalisation.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, method B (20 ppm).

Water (2.3.43). Not more than 0.2 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 35 volumes of *acetonitrile* and 65 volumes of *water.*

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml with the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the same solvent.

Reference solution (a). A solution containing 0.0005 per cent w/v of *modafinil IPRS* and 0.001 per cent w/v of salicylic acid in the solvent mixture.

Reference solution (b). A 0.01 per cent w/v solution of *modafinil IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH2.3 with *orthophosphoric acid* and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

The relative retention time of salicylic acid with reference to modafinil is about 1.1.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to modafinil and salicylic acid is not less than 1.3, the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent, for modafinil peak.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{15}H_{15}NO_2S$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Modafinil Tablets

Modafinil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of modafinil, $C_{15}H_{15}NO_2S$.

Usual strengths. 100 mg; 200 mg.

Identification

Extract a quantity of powdered tablets containing 0.1 g of modafinil with 50 ml each of *dichloromethane* and *water.* Shake and allow the layers to separate. Filter a portion of the lower (dichloromethane) layer, and evaporate to dryness, using a stream of nitrogen if necessary.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *modafinil IPRS* treated in the same manner or with the reference spectrum of modafinil.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 222 nm (2.4.7). Calculate the content of modafinil, $C_{15}H_{15}NO_2S$ in the medium from the absorbance obtained from a solution of known concentration of *modafinil IPRS* in the dissolution medium.

Q. Not less than 75 per cent of the stated amount of $C_{15}H_{15}NO_2S$.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

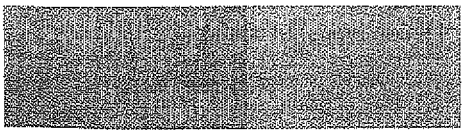
Name	Relative retention time	Correction factor
Modafinil	1.0	---
Salicylic acid A[1]	1.1	---
Modafinil acid B[2]	1.4	1.0
Modafinil sulphone C[3]	1.7	1.1

[1]salicylic acid is used for calculating resolution and is not a potential impurity,

[2]2-[(diphenylmethyl)sulphenyl]acetic acid,

[3]2-[(diphenylmethyl)sulphonyl]acetamide.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to modafinil and salicylic



acid is not less than 1.3. The tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent for modafinil peak.

Inject the test solution. The area of any peak due to modafinil acid and modafinil sulphone is not more than 0.5 per cent each, the area of any other secondary peak is not more than 0.2 per cent and the sum of areas of all the secondary peaks is not more than 1.5 per cent, calculated by area normalisation.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture A. 35 volumes of *acetonitrile* and 65 volumes of *water*.

Solvent mixture B. 35 volumes of *acetonitrile*, 65 volumes of *water* and 1 volume of *acetic acid*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 100 mg of Modafinil to a 250-ml volumetric flask, add 200 ml of solvent mixture B and disperse with the aid of ultrasound for about 5 minutes with intermittent shaking. Dilute with solvent mixture B to volume, mix and filter.

Reference solution (a). A solution containing 0.0005 per cent w/v of *modafinil IPRS* and 0.001 per cent w/v of *salicylic acid IPRS* in solvent mixture A.

Reference solution (b). A 0.04 per cent w/v solution of *modafinil IPRS* in solvent mixture B.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 40°,
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH2.3 with *orthophosphoric acid* and diluting to 1000 ml with *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 5 µl.

The relative retention time with respect to modafinil for salicylic acid is about 1.1.

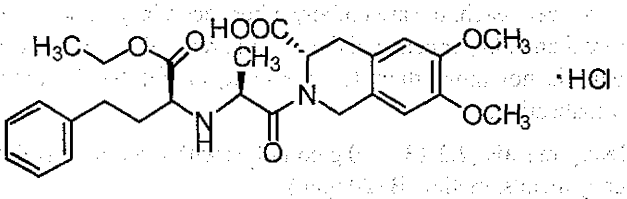
Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to modafinil and salicylic acid is not less than 1.3 in the chromatogram obtained with reference solution (a), the tailing factor is not more than 1.5 and the relative standard deviation of replicate injections is not more than 2.0 per cent for the modafinil peak in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution.

Calculate the content of $C_{15}H_{15}NO_2S$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Moexipril Hydrochloride



$C_{27}H_{34}N_2O_7,HCl$ Mol Wt. 535.0

Moexipril Hydrochloride is (3*S*)-2-[(2*S*)-2-{[(1*S*)-(1-Ethoxycarbonyl)-3-phenyl]propyl]amino}propanoyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride.

Moexipril Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{27}H_{34}N_2O_7,HCl$, calculated on the anhydrous basis.

Category. Antihypertensive.

Description. A white to off white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *moexipril hydrochloride IPRS* or with the reference spectrum of moexipril hydrochloride.

Tests

Specific optical rotation (2.4.24). +33° to +35°, determined on 1.1 per cent w/v solution in *ethanol* at 23°.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *water* and 50 volumes of *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with solvent mixture.

Reference solution. A 0.00025 per cent w/v solution of *moexipril hydrochloride IPRS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 2.0 g of *sodium perchlorate* in 1000 ml of *water*, add 1.0 ml of *triethylamine*, adjusted to pH 2.5 with *perchloric acid*,

B. *acetonitrile*,

– a gradient programme using the conditions given below,
– flow rate: 1 ml per minute,
– spectrophotometer set at 214 nm,
– injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	60	40
30	40	60
32	65	35
40	65	35

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates , the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

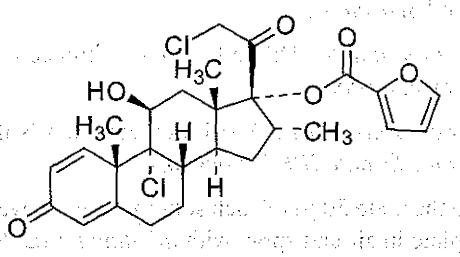
Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Assay. Dissolve 0.5 g in 50.0 ml of *glacial acetic acid* and add 15 ml of 10 percent w/v solution of *mercuric acetate* . Titrate with *0.1 M perchloric acid*, using *crystal-violet* as an indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.053503 g of $C_{27}H_{34}N_2O_7,HCl$.

Mometasone Furoate



$C_{27}H_{30}Cl_2O_6$ Mol. Wt. 521.4

Mometasone Furoate is 9α,21-Dichloro-11β-hydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-17-yl furan-2-carboxylate.

Mometasone Furoate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{27}H_{30}Cl_2O_6$, calculated on the dried basis.

Category. Glucocorticoid.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B, C and D are carried out and tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mometasone furoate IPRS* or with the reference spectrum of mometasone furoate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. a mixture of 1.2 volumes of *water*, 8 volumes of *methanol*, 15 volumes of *ether* and 77 volumes of *dichloromethane*.

Test solution. Dissolve 10 mg of the substance under examination in 10.0 ml of *dichloromethane*.

Reference solution (a). A 0.1 per cent w/v solution of *mometasone furoate IPRS* in *dichloromethane*.

Reference solution (b). Dissolve 10 mg of *beclometasone dipropionate IPRS* in 10.0 ml of reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). Spray with *ethanolic sulphuric acid*. Heat at 120° for 10 minutes or until the spots appear. Allow to cool and examine in daylight and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a). In the chromatogram obtained with reference solution (b), shows 2 spots which, when examined under ultraviolet light at 365 nm, may not be completely separated.

C. Shake about 2 mg with 2 ml of *sulphuric acid* for 15 minutes, a light yellow colour develops. When examined under ultraviolet light at 365 nm, no fluorescence is seen. Add this solution to 10 ml of *water* and mix. The colour fades and there is no fluorescence.

D. Mix 80 mg with 0.3 g of *anhydrous sodium carbonate* and ignite in a crucible until an almost white residue is obtained. Allow to cool and dissolve the residue in 5 ml of *dilute nitric acid*, filter. To 1 ml of the filtrate, add 1 ml of *water*. The solution gives reaction (A) of chlorides (2.3.1).

Tests

Specific optical rotation (2.4.22). + 50° to + 55°, determined in 0.5 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Solvent mixture. 50 volumes of *acetonitrile,* 50 volumes of *water* and 0.1 volume of *acetic acid*.

Test solution. Dissolve 25 mg of the substance under examination in 4 ml of *acetonitrile* and dilute to 25.0 ml with the solvent mixture.

Reference solution (a). Dissolve 2.5 mg of *mometasone furoate IPRS* and 6 mg of *beclometasone dipropionate IPRS* in 10.0 ml of the solvent mixture. Dilute 2.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 20.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to mometasone furoate and beclometasone dipropionate is not less than 6.0.

Inject reference solution (b) and the test solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent) and the sum of all the secondary peaks is not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.6 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 0.67 g complies with the limit test for heavy metals, Method B (30 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 50 mg in 100.0 ml of *ethanol (95 per cent)*. Dilute 2.0 ml of the solution to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 249 nm (2.4.7). Calculate the content of $C_{27}H_{30}Cl_2O_6$ taking 481 as the specific absorbance at 249 nm.

Mometasone Aqueous Nasal Spray

Mometasone Aqueous Nasal Spray is an aqueous suspension of Mometasone Furoate in a suitable pressurised container fitted with an appropriate nasal delivery system.

The nasal spray complies with the requirements stated under Nasal Preparations and with the following requirements.

Mometasone Aqueous Nasal Spray contains not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of mometasone furoate, $C_{27}H_{30}Cl_2O_6$.

Usual strength. 0.05 per cent w/v.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (d).

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 3 volumes of *methanol* and 97 volumes of *1, 2-dichloroethane*.

Test solution. Dissolve 1 mg of Mometasone Furoate in 4 ml of *acetone*, sonicate and filter. Evaporate the filtrate to dryness and dissolve in 1.0 ml of *acetone*.

Reference solution (a). A 0.002 per cent w/v of solution of *mometasone furoate IPRS* in *acetone*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) with 1.0 ml of *acetone*.

Reference solution (c). Dilute 1.0 ml of reference solution (a) with 3.0 ml of *acetone*.

Reference solution (d). A 0.1 per cent w/v solution of *mometasone furoate IPRS* in *acetone*.

Apply to the plate 50 µl of each solution. After development, dry the plate in air and spray with *alkaline tetrazolium blue solution* and heat at 50° for 5 minutes and allow to cool, again spray with *alkaline tetrazolium blue solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained

with reference solution (a) (2.0 per cent). Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent), any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.5 per cent).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a quantity of nasal spray containing1 mg of Mometasone Furoate in 20 ml of hot *methanol (90 per cent)*, and add 25 ml of *2,2,4- trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80 per cent)*. Repeat the extraction of the *2,2,4-trimethylpentane* layer with two further 10 ml quantities of *methanol (80 per cent)*, filtering the extracts through the absorbent cotton. Combine the extracts and add sufficient *methanol (80 per cent)* to produce 50 ml. Filter through a 0.45 µm nylon filter.

Reference solution. A 0.002 per cent w/v solution of *mometasone furoate IPRS* in *methanol (80 per cent)*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with base-deactivated end-capped octadecylsilane bonded to porous silica (5 µm),
- column temperature: 60°,
- mobile phase: a mixture of 45 volumes of *water* and 55 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the peak due to mometasone furoate is not more than 1.2.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{30}Cl_2O_6$.

Labelling. The label states the amount of active ingredient delivered by each actuation of the valve and the number of deliveries available from the container.

Mometasone Cream

Mometasone Furoate Cream

Mometasone Cream contains Mometasone Furoate in a suitable cream base.

Mometasone Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mometasone furoate, $C_{27}H_{30}Cl_2O_6$.

Usual strength. 0.1 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane*.

Test solution. Disperse a quantity of the cream containing about 0.5 mg of Mometasone Furoate in 20 ml of *methanol (80 per cent)* by heating on a water-bath until the solution begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *dichloromethane*, shake vigorously, allow the layers to separate, evaporate the *dichloromethane* layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 ml of *dichloromethane*.

Reference solution (a). A 0.025 per cent w/v solution of *mometasone furoate IPRS* in *dichloromethane*.

Reference solution (b). A mixture of equal volume of test solution and reference solution (a).

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and, while hot, spray with *alcoholic sulphuric acid solution* and heat at 105° for a further 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Creams.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the cream containing about 1 mg of Mometasone Furoate in 20 ml of hot *methanol (90 per cent)*, add 25 ml of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80 per cent)*. Repeat the extraction of the *2,2,4- trimethylpentane* layer with two further 10 ml quantities of *methanol (80 per cent)*, filter the extracts and dilute to 50 ml with *methanol (80 per cent)*, filter.

Reference solution. A 0.002 per cent w/v solution of *mometasone furoate IPRS* in *methanol (80 per cent)*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with base deactivated endcapped octadecylsilane bonded to porous silica (5 µm) (such as Hypersil BDS C18),

- column temperature: 60°,
- mobile phase: a mixture of 45 volumes of *water* and 55 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is not more than 1.2.

Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the cream.

Storage. Store protected from light.

Mometasone Ointment

Mometasone Furoate Ointment

Mometasone Ointment contains Mometasone Furoate in a suitable base.

Mometasone Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mometasone furoate, $C_{27}H_{30}Cl_2O_6$.

Usual strength. 0.1 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *absolute ethanol*, 10 volumes of *acetone* and 100 volumes of *dichloromethane*.

Test solution. Disperse a quantity of the ointment containing about 0.5 mg of Mometasone Furoate in 20 ml of *methanol (80 per cent)* by heating on a water-bath until the solution begins to boil. Shake vigorously, cool in ice for 30 minutes and centrifuge. Mix 10 ml of the supernatant liquid with 3 ml of *water* and 5 ml of *dichloromethane*, shake vigorously, allow the layers to separate, evaporate the *dichloromethane* layer to dryness in a current of nitrogen with gentle heating and dissolve the residue in 1 ml of *dichloromethane*.

Reference solution (a). A 0.025 per cent w/v solution of *mometasone furoate IPRS* in *dichloromethane*.

Reference solution (b). A mixture of equal volume of test solution and reference solution (a).

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and, while hot, spray with *alcoholic sulphuric acid solution* and heat at 105° for a further 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under Ointments.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the ointment containing about 1 mg of Mometasone Furoate in 20 ml of hot *methanol (90 per cent)*, add 25 ml of *2,2,4-trimethylpentane*, cool, shake the mixture and filter the lower methanol layer through a small plug of absorbent cotton previously washed with *methanol (80 per cent)*. Repeat the extraction of the *2,2,4- trimethylpentane* layer with two further 10 ml quantities of *methanol (80 per cent)*, filter the extracts and dilute to 50 ml with *methanol (80 per cent)*, filter.

Reference solution. A 0.002 per cent w/v solution of *mometasone furoate IPRS* in *methanol (80 per cent)*.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Hypersil BDS C18),
- column temperature: 60°,
- mobile phase: a mixture of 45 volumes of *water* and 55 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is not more than 1.2.

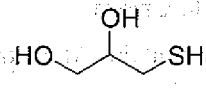
Inject the reference solution and the test solution.

Calculate the content of $C_{27}H_{30}Cl_2O_6$ in the ointment.

Storage. Store protected from light.

Monothioglycerol

Thioglycerol



$C_3H_8O_2S$ Mol. Wt. 108.2

Monothioglycerol is 3-mercaptopropane-1,2-diol.

Monothioglycerol contains not less than 97.0 per cent and not more than 101.0 per cent of $C_3H_8O_2S$, calculated on the anhydrous basis.

Category. Pharmaceutical aid.

Description. A colourless to pale yellow, viscous liquid; hygroscopic.

Tests

pH (2.4.24). 3.5 to 7.0, determined in a 10.0 per cent w/v solution.

Relative density (2.4.29). 1.241 to 1.250.

Refractive index (2.4.27). 1.521 to 1.526 at 25°.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

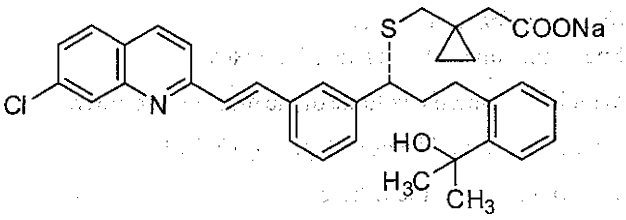
Water (2.3.43). Not more than 5.0 per cent, determined by Method 2 on 50.0 g.

Assay. Weigh 0.2 g, dissolve in 50 ml of *water* and titrate with *0.05 M iodine* using 3 ml of *starch solution*, added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 0.01082 g of $C_3H_8O_2S$.

Storage. Store protected from light and moisture.

Montelukast Sodium



$C_{35}H_{35}ClNNaO_3S$ Mol. Wt. 608.2

Montelukast sodium is monosodium salt of 1-[[[(1*R*)-1-[3-[(1*E*)-2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropaneacetic acid.

Montelukast Sodium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{35}H_{35}ClNNaO_3S$, calculated on the anhydrous basis.

Category. Antiasthmatic (add-on therapy for mild to moderate asthma).

Description. A white to pale yellow powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum obtained with *montelukast sodium IPRS* or with the reference spectrum of montelukast sodium.

B. It gives reaction (A) of sodium salt (2.3.1).

Tests

Specific optical rotation (2.4.22). +95.0° to +106.0°, determined on 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solution, sonicate in ice-cold water.

Solvent mixture. 20 volumes of *water* and 80 volumes of *methanol*.

Test solution. Dissolve about 100 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *montelukast sodium IPRS* in the solvent mixture.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Dilute 3.0 ml of the solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: A. dissolve 3.85 g of *ammonium acetate* in 1000 ml of *water*, add 1 ml of *triethylamine*, adjusted to pH 5.5 with *glacial acetic acid*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	40	60
20	30	70
45	20	80
55	15	85
60	15	85
65	40	60
70	40	60

The relative retention time with reference to montelukast for montelukast sulphoxide isomers is about 0.66 and 0.69 and for montelukast styrene is about 1.38.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the sum of areas of montelukast sulphoxide isomers is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of the peak due to montelukast styrene is not more than area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent) and the sum of areas of all the secondary peaks is not more than 3.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Heavy Metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solution, sonicate in ice-cold water.

Solvent mixture. 20 volumes of *water* and 80 volumes of *methanol*.

Test solution. Dissolve about 50 mg of the substance under examination in 100.0 ml of the solvent mixture.

Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *montelukast sodium IPRS* in the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 22 volumes of buffer solution prepared by dissolving 3.85 g of *ammonium acetate* in 1000 ml of *water*, add 1 ml of *triethylamine*, adjusted to pH 5.5 with *glacial acetic acid* and 78 volumes of *methanol*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the theoretical plates is not less than 2000, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{35}H_{35}ClNNaSO_3$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Montelukast Granules

Montelukast Sodium Granules

Montelukast Granules contain not less than 94.0 per cent and not more than 105.0 per cent of the stated amount of montelukast, $C_{35}H_{36}ClNO_3S$.

Usual strength. 4 mg per pack.

Identification

A. Shake a quantity of the granules containing about 5 mg of montelukast in 150 ml of a solution of 1 volume of *water* and 3 volumes of *methanol* (solvent A). Mix with the aid of ultrasound for 70 minutes with occasional shaking. Add sufficient solvent A to obtain a solution containing 0.0025 per cent w/v of montelukast, mix and centrifuge. When examined in the range 210 to 400 nm (2.4.7), exhibits maxima at 284 nm, 328 nm, 345 nm and 359 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of a 0.5 per cent w/v solution of *sodium dodecyl sulphate* in *water*,
Speed and time. 50 rpm and 20 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. A 0.025 per cent w/v solution of *montelukast sodium IPRS* in *methanol*. Dilute 1.0 ml of the solution to 50.0 ml with 0.5 per cent w/v solution of *sodium dodecyl sulphate*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with phenylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: equal volumes of a 0.2 per cent v/v solution of *trifluoroacetic acid* in *water* and a 0.2 per cent v/v solution of *trifluoroacetic acid* in *acetonitrile*.
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 389 nm,
- injection volume: 25 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{35}H_{36}ClNO_3S$ in the dissolution medium.

1 mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

Q. Not less than 80 per cent of the stated amount of $C_{35}H_{36}ClNO_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 1 volume of *water* and 3 volumes of *methanol.*

Test solution. To a quantity of the granules containing 60 mg of montelukast add 250 ml of solvent mixture and mix with the aid of ultrasound.

Reference solution (a). Dilute 1.0 ml of test solution to 100.0 ml with the solvent mixture. Further dilute 1.0 ml of the solution to 5.0 ml with solvent mixture.

Reference solution (b). To 10 ml of test solution add 4 µl of *hydrogen peroxide solution (100 vol)* and mix. Expose the solution to ambient light for 1 hour (generation of impurity G).

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with phenylhexylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: A. a solution containing 0.2 per cent v/v of *trifluoroacetic* acid in *water,*
  B. a mixture of 2 volumes of *acetonitrile* and 3 volumes of *methanol,*
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 255 nm,
- injection volume: 15 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	48	52
5	48	52
10	45	55
17	45	55
27	25	75
28	25	75
30	48	52
40	48	52

Name	Relative retention time
Montelukast impurity C[1]	0.45
Montelukast impurity G[2]	0.92
Montelukast (Retention time: about 20 minutes)	1.0
Montelukast impurity F[3]	1.04
Montelukast impurity D[4]	1.16
Montelukast impurity E[5]	1.18
Montelukast impurity B[6]	1.55

[1][1-[[[1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfinyl]methyl] cyclopropyl] acetic acid,

[2][1-[[[(1*R*)-1-[3-[(*Z*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl] cyclopropyl]acetic acid,

[3][1-[[[(1*R*)-3-(2-acetylphenyl)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid,

[4][1-[[[(1*R*)-1-[3-[(1*R*)-1-[[[1-(carboxymethyl)cyclopropyl]methyl] sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid,

[5][1-[[[(1*R*)-1-[3-[(1*S*)-1-[[[1-(carboxymethyl)cyclopropyl]methyl] sulfanyl]-2-(7-chloroquinolin-2-yl)ethyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid,

[6][1-[[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(1-methylethenyl)phenyl]propyl]sulfanyl]methyl]cyclopropyl]acetic acid.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to montelukast impurity G and montelukast is not less than 1.5.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to montelukast impurity C is not more than 8.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.7 per cent), the area of the peak corresponding to montelukast impurity B is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent), the area of the peak corresponding to montelukast impurity D and E are not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent), the area of the peak corresponding to montelukast impurity F or G are not more than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent of each), The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), The sum of areas of all the secondary peaks is not more than 13.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.7 per cent). Ignore any peak with an area less than half the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Uniformity of content. Complies with the test stated under Granules.

Determine by liquid chromatography (2.4.14).

Test solution. To the contents of one packet of granules add 100 ml of *methanol* and mix with the aid of ultrasound. Add sufficient *methanol* to obtain a solution containing the equivalent of 0.002 per cent w/v of montelukast.

Reference solution. A 0.002 per cent w/v solution of *montelukast sodium IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 10 cm x 3.0 mm, packed with phenylsilane bonded to porous silica (5 µm),
- column temperature: 50°,
- mobile phase: equal volumes of a 0.2 per cent v/v solution of *trifluoroacetic acid* in *water* and a 0.2 per cent v/v solution of *trifluoroacetic acid* in *acetonitrile*,
- flow rate: 0.9 ml per minute,
- spectrophotometer set at 389 nm,
- injection volume: 5 µl.

Inject the reference solution and the test solution.

Calculate the content of $C_{35}H_{36}ClNO_3S$ in the tablet.

1 mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

Other tests. Comply with the tests stated under Granules.

Assay. Determine by liquid chromatography (2.4.14). as described under Related substances, using the following modifications.

Solvent mixture. A mixture of 1 volume of *water* and 3 volumes of *methanol*.

Test solution. To a quantity of the granules containing 60 mg of Montelukast add sufficient solvent mixture to obtain a solution containing the equivalent of 0.024 per cent w/v of Montelukast, mix with the aid of ultrasound and filter .

Reference solution (a). A 0.025 per cent w/v solution of *montelukast sodium IPRS* in the solvent mixture.

Reference solution (b). To 10 ml of test solution add 4 µl of *hydrogen peroxide solution (100 vol)* and mix. Expose the solution to ambient light for 1 hour (generation of impurity G).

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to montelukast impurity G and montelukast is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{35}H_{36}ClNO_3S$ in the tablets.

1 mg of $C_{47}H_{59}ClN_2O_3S$ is equivalent to 0.7637 mg of $C_{35}H_{36}ClNO_3S$.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of montelukast.

Montelukast Tablets

Montelukast Sodium Tablets

Montelukast Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of montelukast, $C_{35}H_{36}ClNO_3S$.

Usual strengths. 5 mg; 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 0.5 per cent w/v solution of *sodium dodecyl sulphate* in *water*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. Dissolve a quantity of *montelukast sodium IPRS* in the dissolution medium and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.

Use the chromatographic system as described in the Assay.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{35}H_{36}ClNO_3S$ in the tablet.

Q. Not less than 70 per cent of the stated amount of $C_{35}H_{36}ClNO_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.1 per cent v/v solution of *triethylamine* in *methanol*.

Test solution. Weigh and transfer intact tablets containing about 100 mg of montelukast in 200-ml volumetric flask. Add

about 20 ml of *water*, sonicate. Add 150 ml of the solvent mixture and sonicate for 20 minutes at a temperature not exceeding 10° and dilute to 200.0 ml with the solvent mixture, filter.

Reference solution (a). A 0.0025 per cent w/v solution of *montelukast sodium IPRS* in the solvent mixture

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature: 40°,
- mobile phase: A. a solution containing 6.0 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 5.5 with *acetic acid*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	50	50
15	35	65
40	30	70
60	20	80
65	20	80
70	50	50
75	50	50

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to sulphoxide impurity at relative retention time 0.63 is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), and the area of the peak corresponding to styrene impurity at about relative retention time 1.37 is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.1 per cent v/v solution of *triethylamine* in *methanol*.

Test solution. Weigh and transfer intact tablets containing about 100 mg of montelukast in 200-ml volumetric flask. Add about 20 ml of *water*, sonicate. Add 150 ml of the solvent mixture, sonicate for 20 minutes at a temperature not exceeding 10°. Dilute to 200.0 ml with the solvent mixture, filter. Dilute 5.0 ml of the solution to 25.0 ml with the solvent mixture.

Reference solution. Dissolve 26 mg of *montelukast sodium IPRS* in 250.0 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature: 40°,
- mobile phase: a mixture of 20 volumes of a buffer solution prepared by dissolving 3.85 g of *ammonium acetate* in 1000 ml of *water*, add 1.0 ml *triethylamine*, adjusted to pH5.5 with *acetic acid* and 80 volumes of *methanol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent

Inject the reference solution and the test solution.

Calculate the content of $C_{35}H_{36}ClNO_3S$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of montelukast.

Montelukast and Levocetirizine Tablets

Montelukast Sodium and Levocetirizine Hydrochloride Tablets

Montelukast and Levocetirizine Tablets contain montelukast sodium equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of montelukast, $C_{35}H_{36}ClNO_3S$ and levocetirizine hydrochloride, $C_{21}H_{25}N_2O_3Cl,2HCl$.

Usual strengths. Montelukast, 5 mg and Levocetirizine Hydrochloride, 2.5 mg; Montelukast, 4 mg and Levocetirizine Hydrochloride, 2.5 mg; Montelukast, 10 mg and Levocetirizine Hydrochloride, 5 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of 1.0 per cent w/v *sodium lauryl sulphate* in *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate with mobile phase A.

Reference solution (a). A 0.057 per cent w/v solution of *montelukast sodium IPRS* in *methanol.*

Reference solution (b). A 0.028 per cent w/v solution of *levocetirizine hydrochloride IPRS* in *methanol.*

Reference solution (c). Dilute a suitable quantity of reference solution (a) and (b) with mobile phase A to obtain a solution having similar concentration as that of test solution.

Use chromatographic system as described under Assay, using 100 µl injection volumes.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{35}H_{36}ClNO_3S$ and $C_{21}H_{25}N_2O_3Cl,2HCl$.

Q. Not less than 70 per cent of the stated amounts of $C_{35}H_{36}ClNO_3S$ and $C_{21}H_{25}N_2O_3Cl,2HCl$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 15 volumes of mobile phase A and 85 volumes of *methanol.*

NOTE — Carry out the test protected from light and prepare solution immediately before use.

Test solution. Disperse a quantity of powdered tablets containing 25 mg of Levocetirizine in 30 ml of solvent mixture with the aid of ultrasound for 5 minutes, and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.005 per cent w/v of *montelukast sulphoxide IPRS* and *montelukast styrene IPRS* in the solvent mixture.

Reference solution (b). A solution containing 0.053 per cent w/v of *montelukast sodium IPRS* and 0.025 per cent w/v of *levocetrizine hydrochloride IPRS* in the solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution (c). Dilute 5.0 ml of reference solution (a) and reference solution (b) to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS),
- column temperature: 40°,
- sample temperature: 8°,
- mobile phase: A. a 0.6 per cent w/v solution of *ammonium acetate* in *water*, adjusted to pH 5.5 with *glacial acetic acid*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	48	52
15	48	52
38	15	85
55	15	85
60	48	52
65	48	52

Name	Relative retention time
Levocetirizine	0.24
Montelukast sulphoxide impurity	0.89
Montelukast (Retention time about 35 minutes)	1.0
Montelukast styrene impurity	1.12

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (c) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to montelukast sulphoxide is not more than the area of the peak due to montelukast sulphoxide in the chromatogram obtained with reference solution (c) (2.0 per cent), the area of any peak corresponding to montelukast styrene is not more than 0.5 times the area of the peak due to montelukast styrene in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any other secondary peak is not more than the area of the peak due to montelukast in the chromatogram obtained reference solution (c) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 4 times the area of the peak due to montelukast in the chromatogram obtained with reference

solution (c) (4.0 per cent). Ignore any peak with an area less than 0.05 times the area of the peak due to montelukast in the chromatogram obtained with reference solution (c) (0.05 per cent) and the peak due to levocetirizine.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic system and reference solution (c) as described under Assay.

Test solution. Disperse one tablet in 100-ml of volumetric flask. Add 25 ml of solvent mixture and sonicate for about 10 minutes with intermittent shaking. Dilute to volume with solvent mixture, mix and centrifuge.

Inject reference solution (c) and the test solution.

Calculate the contents of $C_{35}H_{36}ClNO_3S$ and $C_{21}H_{25}N_2O_3Cl,2HCl$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Solvent mixture. 30 volumes of a buffer solution prepared by dissolving 0.7791 g of *ammonium acetate* in 1000 ml of *water* and add 0.1 ml of *glacial acetic acid* and 70 volumes *acetonitrile*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing 25 mg of Montelukast Sodium in a 100-ml volumetric flask, add 70.0 ml of solvent mixture and sonicate for 15 minutes and make to volume with solvent mixture, mix and centrifuge. Dilute 5.0 ml of the solution to 25.0 ml with the solvent mixture.

Reference solution (a). A 0.25 per cent w/v solution of *montelukast sodium IPRS* in the solvent mixture.

Reference solution (b). A 0.125 per cent w/v solution of *levocetirizine hydrochloride IPRS* in the solvent mixture.

Reference solution (c). Dilute reference solution (a) and (b) with the Solvent mixture to obtain a solution having a known concentration similar to the test solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase A. a mixture of 60 volumes of a buffer solution prepared by dissolving 0.7791 g of *ammonium acetate* in 1000 ml of *water* and add 0.1 ml of *glacial acetic acid* and 40 volumes *acetonitrile*.

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
7	100	0
8	55	45
24	55	45
25	10	90
29	10	90
29.1	100	0
35	100	0

Inject reference solution (c). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

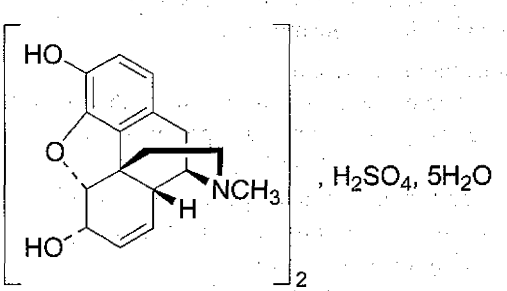
Inject reference solution (c) and the test solution.

Calculate the contents of $C_{35}H_{36}ClNO_3S$ and $C_{21}H_{25}N_2O_3Cl$, 2HCl in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of montelukast and levocetirizine hydrochloride.

Morphine Sulphate



$(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ Mol. Wt. 758.8

Morphine Sulphate is 7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol sulphate pentahydrate.

Morphine Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{17}H_{19}NO_3)_2,H_2SO_4$, calculated on the dried basis.

Category. Narcotic analgesic.

Description. White, acicular crystals or cubical masses or a white, crystalline powder.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.015 per cent w/v solution shows an absorption maximum only at about 285 nm; absorbance at about 285 nm, about 0.65.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorption maximum only at about 298 nm; absorbance at about 298 nm, about 0.34.

C. Add a few mg of the powdered substance to a mixture of 1 ml of *sulphuric acid* and 0.05 ml of *formaldehyde solution*; a purple colour is produced.

D. Dissolve 5 mg in 5 ml of *water* and add 0.15 ml of *dilute potassium ferricyanide solution* and 0.05 ml of *ferric chloride solution*; a bluish green colour is produced immediately, which changes rapidly to blue.

E. It gives the reactions of sulphates (2.3.1).

Tests

Acidity. Dissolve 0.2 g in 10 ml of freshly boiled and cooled *water* and titrate with *0.02 M sodium hydroxide* using *methyl red solution* as indicator. Not more than 0.2 ml of *0.02 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 1.0 per cent v/v solution of *acetic acid.*

Test solution. Dissolve 125 mg of the substance under examination in 50 ml of the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100 ml with the solvent mixture. Dilute 2.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature: 35°,
- mobile phase: A. a 0.1 per cent w/v solution of *sodium heptanesulphonate*, adjusted to pH 2.6 with *orthophosphoric acid*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
2	85	15
35	50	50
40	50	50
42	85	15

Name	Relative retention time	Correction factor
Morphine impurity F[1]	0.95	—
Morphine (Retention time: about 12.5 minutes)	0.32	---
Morphine impurity E[2]	1.1	0.5
Morphine impurity C[3]	1.6	0.4
Morphine impurity B[4]	1.9	0.25

[1](17*S*)-7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol 17-oxide (morphine *N*-oxide),

[2]7,8-didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6-one (morphinone),

[3](6,7,8,14-tetradehydro-4,5α-epoxy-6-methoxy-17-methylmorphinan-3-ol (oripavine),

[4]7,7',8,8'-tetradehydro-4,5α:4',5'α-diepoxy-17,17'-dimethyl-2,2'-bimorphinanyl-3, 3',6α,6'α-tetrol (2,2'-bimorphine).

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of the peak corresponding to 2,2'-bimorphine (morphine impurity B) is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.4 per cent). The area of the each peak corresponding to oripavine (morphine impurity C) and morphinone (morphine impurity E) is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent) and sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 9.0 to 12.0 per cent, determined on 0.5 g by drying in an oven at 145° for 1 hour.

Assay. Weigh 0.5 g, dissolve in 30 ml of *glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.06688 g of $(C_{17}H_{19}NO_3)_2, H_2SO_4$.

Storage. Store protected from light and moisture.

Morphine and Atropine Injection

Morphine Sulphate and Atropine Sulphate Injection

Morphine and Atropine Injection is a sterile isotonic solution in Water for Injections containing 1.0 per cent w/v of Morphine Sulphate and 0.06 per cent w/v of Atropine Sulphate.

Morphine and Atropine Injection contains not less than 0.90 per cent w/v and not more than 1.10 per cent w/v of morphine sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$, and not less than 0.054 per cent w/v and not more than 0.066 per cent w/v of atropine sulphate, $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

Usual Strengths. Morphine sulphate 1 per cent and atropine sulphate 0.06 per cent.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution*.

Test solution. Add 1 ml of *5 M ammonia* to 1 ml of the injection, extract with two quantities, each of 5 ml, of *chloroform*, filter the combined extracts through *anhydrous sodium sulphate*, evaporate to dryness in a current of warm air and dissolve the residue in 0.5 ml of *chloroform*.

Reference solution (a). Treat 1 ml of a 0.06 per cent w/v solution of *atropine sulphate IPRS* in the same manner as for the test solution.

Reference solution (b). Treat 1 ml of a 1 per cent w/v solution of *morphine sulphate IPRS* in the same manner as for the test solution.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution*. The principal spots in the chromatogram obtained with the test solution correspond to the spots in the chromatograms obtained with reference solution (a) and (b).

Tests

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *For atropine sulphate* — To 10.0 ml add 10 ml of *water* and 5 ml of *1 M sodium hydroxide* and extract successively with 15, 10 and 10 ml of *chloroform* and continue the extraction with 10-ml quantities of *chloroform* until complete extraction of alkaloids has been effected (2.6.4). Wash the chloroform extracts with the same 5 ml of *water* (preserve the aqueous solution and the washings for the Assay for morphine sulphate). Evaporate the chloroform, dissolve the residue in 2 ml of *ethanol (95 per cent)*, add 2.0 ml of *0.025 M sulphuric acid*, cool and titrate the excess of acid with *0.05 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.025 M sulphuric acid* is equivalent to 0.01737 g of $(C_{17}H_{23}NO_3)_2,H_2SO_4,H_2O$.

For morphine Sulphate — Combine the aqueous solution and washings obtained in the Assay for atropine sulphate, add 1 g of *ammonium sulphate* and 25 ml of *ethanol (95 per cent)* and extract with 40 ml, followed by successive quantities of 40, 20 and 20 ml, of a mixture of 1 volume of *ethanol (95 per cent)* and 3 volumes of *chloroform*, washing each extract with the same two successive quantities, each of 5 ml, of *water* and continue the extraction until complete extraction of alkaloids has been effected (2.6.4). Evaporate the chloroform, boil the residue with 10.0 ml of *0.05 M sulphuric acid*, cool and titrate the excess of acid with *0.1 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.03794 g of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

Storage. Store protected from light.

Morphine Injection

Morphine Sulphate Injection

Morphine Injection is a sterile solution of Morphine Sulphate in Water for Injections.

Morphine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of morphine sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

Usual strength. 10 mg per ml; 15 mg per ml; 50 ml per ml.

Identification

A. Evaporate a volume containing 5 mg of Morphine Sulphate to dryness on a water-bath. Dissolve the residue in 5 ml of *water* and add 0.15 ml of *dilute potassium ferricyanide solution*; a bluish green colour is produced immediately, which changes rapidly to blue.

B. It gives reaction (A) of sulphates (2.3.1).

Tests

pH (2.4.24). 2.5 to 6.0.

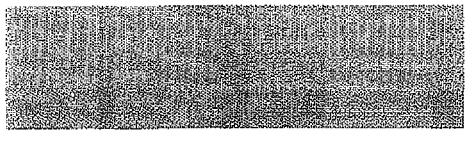
Bacterial endotoxins (2.2.3). Not more than 17.0 Endotoxin Units per mg of morphine; if labelled for intrathecal use it contains not more than 14.29 Endotoxin Units per mg of morphine.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Measure a volume containing 25 mg of Morphine Sulphate and dilute with sufficient of the mobile phase to produce 100.0 ml, freshly prepared.

Reference solution. Weigh 25 mg of *morphine sulphate IPRS* and dissolve in sufficient of the mobile phase to produce 100.0 ml, freshly prepared.



Chromatographic system
- a stainless steel column 40 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 0.73 g of *sodium heptanesulphonate* in 720 ml of *water*, add 280 ml of *methanol* and 10 ml of *glacial acetic acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 20 µl.

Inject the test solution and reference solution.

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in the injection.

Storage. Store protected from light.

Morphine Tablets

Morphine Sulphate Tablets

Morphine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of morphine sulphate, $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

Usual strengths. 10 mg; 20 mg; 30 mg; 60 mg.

Identification

A. Disperse a quantity of the powdered tablets containing 20 mg of Morphine Sulphate with 5 ml of *water*, filter and add to the filtrate 0.05 ml of *iron(III) chloride solution*; a blue colour is produced.

B. Shake a quantity of the powdered tablets containing 10 mg of Morphine Sulphate with 10 ml of *water*, filter and to 5 ml of the filtrate add 0.15 ml of *dilute potassium hexacyanoferrate(III) solution* and 0.05 ml of *iron(III) chloride solution*; a bluish green colour is produced immediately, which changes rapidly to blue.

C. It gives the reactions of sulphates (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *phosphate buffer pH 6.5*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with *phosphate buffer pH 6.5*, to produce a solution containing 0.001 per cent w/w of Morphine Sulphate.

Reference solution. A 0.001 per cent w/v solution of *morphine sulphate IPRS* in *phosphate buffer pH 6.5*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of *0.01M sodium heptanesulphonate* in *0.1M acetic acid*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 211 nm,
- injection volume: 50 µl.

Inject the reference solution and the test solution.

Calculate the content of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$ in the medium.

1 g of $(C_{17}H_{19}NO_3)_2$ is equivalent to 1.330 g of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

Q. Not less than 70 per cent of the stated amount of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O_4$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 2.5 volumes of *13.5 M ammonia*, 32.5 volumes of *acetone*, 35 volumes of *ethanol (70 per cent)* and 35 volumes of *toluene*.

Solvent mixture. Equal volumes of *ethanol (95 per cent)* and *water*

Test solution. Disperse a quantity of the powdered tablets containing 10 mg of Morphine Sulphate in 10 ml of the solvent mixture for 10 minutes and filter.

Reference solution (a). Dissolve 5 mg of *codeine phosphate* in 5 ml of the test solution and dilute 1.0 ml of the solution to 200.0 ml with the solvent mixture.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 5.0 ml with the solvent mixture.

Apply to the plate 50 µl of each solution. After development, dry the plate in air and spray the plate with *potassium iodobismuthate solution*, and allow it to dry for 15 minutes in air and spray with *hydrogen peroxide solution (10 volumes)*. The spot corresponding to codeine is bluish-grey and the spot corresonding to morphine is pinkish. In the chromatogram obtained with the test solution, any spot corresponding to codeine is not more intense than the spot due to codeine in the chromatogram obtained with reference solution (a) (0.5 per cent), any other secondary spot is not more intense than the spot corresponding to morphine in the chromatogram obtained with reference solution (a) (0.5 per cent) and not more than two such spots are more intense than the spot corresponding to morphine in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (a) shows two clearly separated spots. Ignore any spot with an R_f value of less than 0.1.

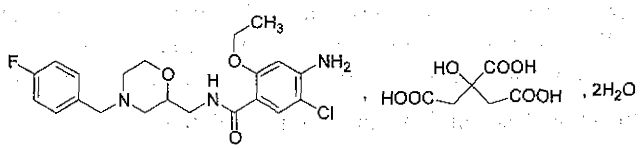
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 0.1 g of Morphine Sulphate with 25 ml of *water* and 5 ml of *1M sodium hydroxide*, add 1 g of *ammonium sulphate*, shake to dissolve, add 20 ml of *ethanol (95 per cent)* and extract with successive quantities of 40, 20, 20 and 20 ml of a mixture of 3 volumes of *chloroform* and 1 volume of *ethanol (95 per cent)*. Wash each extract with the same 5 ml of *water*, filter and evaporate the solvent. Dissolve the residue in 10 ml of *0.05 M hydrochloric acid*, boil, cool, add 15 ml of *water*. Titrate the excess of acid with *0.05 M sodium hydroxide* using *methyl red solution* as indicator. Carry out a blank titration.

1 ml of *0.05 M hydrochloric acid* is equivalent to 0.01897 g of $(C_{17}H_{19}NO_3)_2,H_2SO_4,5H_2O$.

Storage. Store protected from light.

Mosapride Citrate Dihydrate



$C_{21}H_{25}ClFN_3O_3,C_6H_8O_7,2H_2O$ Mol. Wt. 650.0

Mosapride Citrate Dihydrate is (*RS*)-4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)-2-morpholinyl]methyl} benzamide citrate dihydrate.

Mosapride Citrate Dihydrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$, calculated on the anhydrous basis.

Category. Gastroprokinetic agent.

Description. A white or yellowish white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mosapride citrate dihydrate IPRS* or with the reference spectrum of mosapride citrate dihydrate.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dissolve 6.5 mg of *citric acid monohydrate* in 100.0 ml of the mobile phase.

Reference solution (b). Dissolve 20 mg of *mosapride citrate dihydrate IPRS* in 100 ml of the mobile phase. Stir with the aid of ultrasound to dissolve, cool and dilute 1 ml of the solution to 200 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 60 volumes of a buffer solution, prepared by dissolving 1.4 ml of *orthophosphoric acid* in 1000 ml of *water*, adjusted to pH 3.0 with *triethylamine*, and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume: 20 µl.

Inject reference solution (b). The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject reference solution (a). Inject the test solution. Measure the responses for all peaks except the peak due to the analyte, peaks from the blank and the peak corresponding to citric acid. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). 5.0 per cent to 7.0 per cent, determined on 1.0 g.

Assay. Weigh 0.35 g, dissolve in 70 ml of a mixture of 1 volume of *glacial acetic acid* and 7 volumes of *methyl ethyl ketone*. Cover the beaker and heat on a water-bath at 80° to dissolve. Cool and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.061405 g of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$.

Storage. Store protected from moisture.

Mosapride Citrate Tablets

Mosapride Citrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mosapride citrate, $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$.

Usual strengths. 2.5 mg; 5 mg; 10 mg; 15 mg.

Identification

In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 500 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, diluted if necessary, with the dissolution medium

Reference solution.

For 2.5 mg tablets—

Weigh 26.5 mg of *mosapride citrate dihydrate IPRS* and dissolve in 25 ml of the mobile phase. Dilute 5 ml of the solution to 1000 ml with the medium.

For other than 2.5mg tablets—

Weigh 26.5 mg of *mosapride citrate dihydrate IPRS* and dissolve in 25 ml of the mobile phase. Dilute 5 ml of the solution to 500 ml with the medium.

Use the chromatographic system described under Assay

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$.

Q. Not less than 70 per cent of the stated amount of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$.

Uniformity of content. Complies with the test stated under tablets.

Determine by liquid chromatography (2.4.14) as described in the Assay using the following solutions.

Test solution. Dissolve 1 tablet in 100 ml of the mobile phase. Centrifuge for 15 minutes. Dilute the clear supernatant liquid if necessary, with the mobile phase to produce a solution containing 0.02 mg of mosapride citrate per ml.

Reference solution. Weigh 26.5 mg of *mosapride citrate dihydrate IPRS* and dissolve in 100 ml of the mobile phase. Dilute with the mobile phase to produce a solution containing of 0.02 mg of mosapride citrate per ml.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 10 mg of mosapride citrate and dissolve in 50 ml of the mobile phase. Centrifuge for 15 minutes. Dilute 5 ml of the clear supernatant liquid to 50 ml with the mobile phase.

Reference solution. Weigh 21.2 mg of *mosapride citrate dihydrate IPRS* and dissolve in 100 ml of the mobile phase. Dilute 5 ml of the solution to 50 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 60 volumes of a buffer solution, prepared by dissolving 1.4 ml of *orthophosphoric acid* in 1000 ml of *water* and adjusted to pH 3.0 with *triethylamine*, and 40 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume: 20 µl.

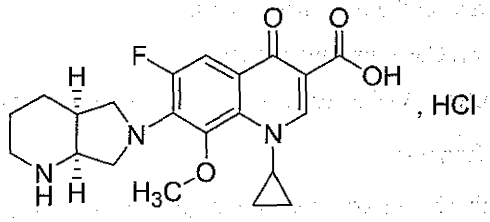
Inject the reference solution. The test is not valid unless the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of $C_{21}H_{25}ClFN_3O_3,C_6H_8O_7$ in the tablets.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous mosapride citrate.

Moxifloxacin Hydrochloride



$C_{21}H_{25}ClFN_3O_4$ Mol. Wt. 437.9.

Moxifloxacin Hydrochloride is 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4as,7as)-octahydro-6*H*-pyrolo'3,4-b] pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride.

Moxifloxacin Hydrochloride contains 98.0 per cent to 102.0 per cent of $C_{21}H_{25}ClFN_3O_4$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A light yellow or yellow powder or crystals, slightly hygroscopic.

Identification

A. Determined by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *moxifloxacin hydrochloride IPRS* or with the reference spectrum of moxifloxacin hydrochloride.

B. Dissolve 50 mg in 5 ml of *water*, add 1 ml of *dilute nitric acid*, mix, allow to stand for 5 minutes and filter. The filtrate gives reactions of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *dilute sodium hydroxide* solution is not more opalescent than standard OS2 and not more intensely coloured than reference solution GYS2 (2.4.1) If intended for use in the manufacture of parenteral preparations, the solution is clear and not more intensely coloured than reference solution GYS2 (2.4.1).

pH (2.4.24). 3.9 to 4.6 , determined in 0.2 per cent w/v solution in *carbon dioxide- free water*.

Specific optical rotation (2.4.22). −138.0° to −125.0°, determined on 1.0 per cent w/v solution in equal mixture of *acetonitrile* and *water*.

Related substances. Determined by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 0.5 g of *tetrabutylamonium hydrogen sulphate* and 1.0 g of *potassium dihydrogen phosphate* in 500 ml of *water*. Add 2 ml of *orthophosphoric acid* and 0.05g of *anhydrous sodium sulphite*, dilute to 1000 ml with *water*.

Test solution (a). Dissolve 50 mg of the substances under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Test solution (b). Dilute 2.0 ml of test solution (a) to 20.0 ml with the solvent mixture.

Reference solution (a). A 0.01 per cent w/v solution of *moxifloxacin hydrochloride IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenylsilane bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: a mixture of 28 volumes of *methanol* and 72 volumes of a solution containing 0.05 per cent w/v of *terabutylammonium hydrogen sulphate* and 0.1 per cent w/v of *potassium dihydrogen phosphate* and 0.34 per cent w/v of *orthophosphoric acid*,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 293 nm,
- injection volume: 10 µl.

Name	Relative retention time	Correction factor
Moxifloxacin (Retention time: about 14 minutes)	1.0	—
Moxifloxacin impurity A[1]	1.1	—
Moxifloxacin impurity B[2]	1.3	1.4
Moxifloxacin impurity C[3]	1.4	—
Moxifloxacin impurity D[4]	1.6	—
Moxifloxacin impurity E[5]	1.7	3.5

[1] 1-cyclopropyl-6,8-difluoro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

[2] 1-cyclopropyl-6,8-dimethoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

[3] 1-cyclopropyl-8-ethoxy-6-fluoro-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

[4] 1-cyclopropyl-8-fluoro-6-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

[5] 1-cyclopropyl-6-fluoro-8-hydroxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailinag factor is not more than 2.0.

Inject reference solution (b) and test solution (a). Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with test solution (a), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 4.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14), as described in the Related substances.

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 50.0 ml with the solvent mixture. Dilute 2.0 ml of the solution to 20.0 ml with the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of *moxifloxacin hydrochloride IPRS* in the solvent mixture.

Inject the reference solution and the test solution.

Calculate the content of $C_{21}H_{25}ClFN_3O_4$.

Storage. Store protected from light and moisture.

Moxifloxacin Eye Drops

Moxifloxacin Eye Drops are a sterile solution of Moxifloxacin Hydrochloride in purified water.

Moxifloxacin Eye Drops Contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of moxifloxacin, $C_{21}H_{24}FN_3O_4$.

Usual strength. 0.5 per cent w/v.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.3 to 7.3.

Related substances. A. Determine by liquid chromatography (2.4.14) as described in the Assay using following modifications.

Inject the test solution. The area of any secondary peak is not more than 0.5 per cent and the sum of areas of all the secondary peaks is not more than 1.5 per cent, calculated by area normalization.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the eye drops containing 5 mg of moxifloxacin to 50.0 ml with mobile phase A.

Reference solution (a). A 0.01 per cent w/v solution of *moxifloxacin hydrochloride IPRS* in mobile phase A.

Reference solution (b). A 0.001 per cent w/v solution of *moxifloxacin impurity A IPRS (1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-[(4aS ,7aS)-octahydro-6H -pyrrolo[3,4-b] pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid IPRS)* in reference solution (a).

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with phenyl groups chemically bonded to porous silica (5 µm),
- column temperature: 45°,
- mobile phase: A. dissolve 0.5 g of *tetrabutylammonium hydrogen sulphate* and 1.0 g of *monobasic potassium phosphate* in 1000 ml of *water*, add 2 ml of *orthophosphoric acid*, filter,

B. *methanol,*

- a gradient programme using the conditions given below,
- spectrophotometer set at 293 nm,
- injection volume: 25 µl.

Time (in min)	Mobile phase A (per cent w/v)	Mobile phase B (per cent v/v)	Flow rate (ml per minute)
0	69	31	0.5
30	69	31	0.5
31	60	40	0.9
36	60	40	0.9
37	69	31	0.5
42	69	31	0.5

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to moxifloxacin and moxifloxacin impurity A is not less than 2.0 in the chromatogram obtained with reference solution (b). The column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{21}H_{24}FN_3O_4$ in the eye drops.

Multiple Electrolytes and Dextrose Injection Type I

Multiple Electrolytes and Dextrose Injection Type I is a sterile solution of Dextrose and suitable salts in Water for Injection to provide sodium, potassium, magnesium, acetate, phosphate and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

Usual strength.

Sodium acetate	0.32 g
Potassium chloride	0.13 g
Dipotassium hydrogen phosphate	0.026 g
Magnesium chloride	0.031 g
Dextrose	5.0 g
Water for Injections to	100 ml
Concentration of electrolytes in mmol / l	
Sodium	23.0
Potassium	20.0
Magnesium	1.5
Acetate	23.0
Chloride	20.0
Phosphate	1.5

Multiple Electrolytes and Dextrose Injection Type I contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, magnesium, Mg, acetate, $C_2H_3O_2$, and phosphate, PO_4. It also contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl and not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides phosphates, sodium salts, potassium salts and magnesium salts (2.3.1).

Tests

pH (2.4.24). 3.0 to 7.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *For sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For total potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For magnesium — To 50.0 ml add 50 ml of *water* and 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.005 M disodium edetate* using 50 mg of *eriochrome black T mixture* as indicator.

1 ml of *0.005 M disodium edetate* is equivalent to 0.1215 mg of Mg.

For acetate — Determine by liquid chromatography (2.4.14).

Test solution. Dilute a measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of sodium acetate.

Reference solution. Dissolve a weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm x 7.8 mm packed with the same column material,
- column temperature: 60°,
- mobile phase: *0.1 M sulphuric acid*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. Calculate the content of acetate in the preparation under examination.

For phosphate — Dilute a measured volume containing about 4 mg of phosphate with sufficient *water* to produce 50.0 ml. Transfer 2.0 ml of the solution to a test-tube. Add 1.0 ml of a 5 per cent w/v solution of *ammonium molybdate* in a cooled mixture of *sulphuric acid* and *water* (15:85) and allow to stand for 3 minutes. Add 1.0 ml of a freshly prepared 0.5 per cent w/v solution of *hydroquinone* containing 1 drop of *sulphuric acid* and 1.0 ml of a freshly prepared 20 per cent w/v solution of *anhydrous sodium sulphite*, add 5.0 ml of *water*, mix and allow to stand for 30 minutes. Measure the absorbance of the resulting solution at the maximum at about 640 nm (2.4.7), using as the blank a solution prepared in the same manner by treating 2 ml of *water* instead of the solution of the preparation under examination. Calculate the content of phosphate from the absorbance obtained by simultaneously carrying out the operation using a known concentration of about 0.11 mg per ml of *dipotassium hydrogen phosphate* in *water* instead of the solution of the preparation under examination.

For total chloride — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To a measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to

produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

Multiple Electrolytes and Dextrose Injection Type II

Multiple Electrolytes and Dextrose Injection Type II is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, calcium, magnesium and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

Usual strength.

Sodium acetate	0.33 g
Sodium chloride	0.088 g
Potassium chloride	0.12 g
Calcium chloride dihydrate	0.037 g
Magnesium chloride	0.031 g
Dextrose	5.0 g
Water for Injections to	100 ml
Concentration of electrolytes in mmol / l	
Sodium	40.0
Potassium	16.0
Calcium	2.5
Chloride	40.0
Magnesium	1.5
Acetate	24.0

Multiple Electrolytes and Dextrose Injection Type II contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, calcium, Ca, magnesium, Mg and acetate, $C_2H_3O_2$. It contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides sodium salts, potassium salts, calcium salts and magnesium salts (2.3.1).

Tests

pH (2.4.24). 3.0 to 7.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For calcium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm and using *calcium solution FP* or *calcium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For magnesium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 296 nm and using *magnesium solution FP* or *magnesium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For acetate — Determine by liquid chromatography (2.4.14).

Test solution. Dilute a measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

Reference solution. Dissolve a weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column temperature: 60°,
- mobile phase: *0.1 M sulphuric acid*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. Calculate the content of acetate in the preparation under examination.

For total chloride — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To a measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

Multiple Electrolytes and Dextrose Injection Type III

Multiple Electrolytes and Dextrose Injection Type III is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, acetate, chloride and phosphate ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

Usual strength.

Sodium acetate	0.28 g
Sodium chloride	0.10 g
Potassium chloride	0.15 g
Dipotassium hydrogen phosphate	0.13 g
Dextrose	5.0 g
Water for Injections to	100 ml
Concentration of electrolytes in mmol / l	
Sodium	37.0
Potassium	35.0
Acetate	20.0
Chloride	37.0
Phosphate	7.5

Multiple Electrolytes and Dextrose Injection Type III contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, acetate, $C_2H_3O_2$, and phosphate, PO_4, and not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides phosphates, sodium salts and potassium salts (2.3.1).

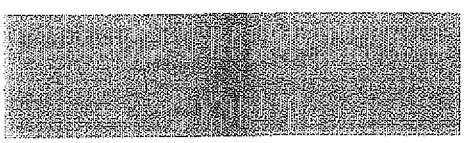
Tests

pH (2.4.24). 3.0 to 7.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).



Assay. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For acetate — Determine by liquid chromatography (2.4.14).

Test solution. Dilute a measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

Reference solution. Dissolve a weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system
- a stainless steel column 30 cm x 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm x 7.8 mm packed with the same column material,
- column temperature: 60°,
- mobile phase: *0.1 M sulphuric acid*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. Calculate the content of acetate in the preparation under examination.

For phosphate — Dilute a measured volume containing about 4 mg of phosphate with sufficient *water* to produce 50.0 ml. Transfer 2.0 ml of the solution to a test-tube. Add 1.0 ml of a 5 per cent w/v solution of *ammonium molybdate* in a cooled mixture of *sulphuric acid* and *water* (15:85) and allow to stand for 3 minutes. Add 1.0 ml of a freshly prepared 0.5 per cent w/v solution of *hydroquinone* containing 1 drop of *sulphuric acid* and 1.0 ml of a freshly prepared 20 per cent w/v solution of *anhydrous sodium sulphite*, add 5.0 ml of *water*, mix and allow to stand for 30 minutes. Measure the absorbance of the resulting solution at the maximum at about 640 nm (2.4.7), using as the blank a solution prepared in the same manner by treating 2 ml of *water* instead of the solution of the preparation under examination. Calculate the content of phosphate from the absorbance obtained by simultaneously carrying out the operation using a known concentration of about 0.11 mg per ml of *dipotassium hydrogen phosphate* in *water* instead of the solution of the preparation under examination.

For total chloride — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To a measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

Multiple Electrolytes and Dextrose Injection Type IV

Multiple Electrolytes and Dextrose Injection Type IV is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, ammonium and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

Usual strength.

Sodium chloride	0.37	g
Potassium chloride	0.13	g
Ammonium chloride	0.37	g
Dextrose	5.0	g
Water for Injections to	100	ml
Concentration of electrolytes in mmol / l		
Sodium	63.0	
Potassium	17.0	
Ammonium	70.0	
Chloride	150.0	

Multiple Electrolytes and Dextrose Injection Type IV contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, and ammonium, NH_4 and not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of chlorides ammonium salts, sodium salts and potassium salts (2.3.1).

Tests

pH (2.4.24). 3.0 to 7.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm: absorbance at about 284 nm, not more than 0.25 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For ammonium — Transfer a measured volume of the preparation under examination, containing about 63 mg of ammonium, to a 500-ml Kjeldahl flask, dilute to 200 ml with *water*, mix and add 50 ml of 40 per cent w/v solution of *sodium hydroxide*. Connect the flask immediately to a well-cooled condenser through a distillation trap. Let the delivery tube from the condenser dip into 40 ml of a 4.0 per cent w/v solution of *boric acid* contained in a suitable receiver. Heat to boiling and distil about 200 ml. Cool the liquid in the receiver, if necessary, and titrate with *0.05 M sulphuric acid* using *methyl red solution* as indicator. Carry out a blank titration.

1 ml of *0.05 M sulphuric acid* is equivalent to 1.804 mg of ammonium, NH_4.

For total chloride — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To a measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

Multiple Electrolytes and Dextrose Injection Type V

Multiple Electrolytes and Dextrose Injection Type V is a sterile solution of Dextrose and suitable salts in Water for Injections to provide sodium, potassium, calcium, magnesium, acetate, citrate and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

Usual strength.

Sodium acetate	0.64 g
Sodium chloride	0.50 g
Sodium citrate	0.075 g
Potassium chloride	0.075 g
Calcium chloride	0.035 g
Magnesium chloride	0.031 g
Dextrose	5.0 g
Water for Injections to	100 ml

Concentration of electrolytes in mmol / l	
Sodium	140.0
Potassium	10.0
Calcium	2.5
Magnesium	1.5
Acetate	47.0
Chloride	103.0
Citrate	2.5

Multiple Electrolytes and Dextrose Injection Type V contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K and not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It also contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. 20 ml gives the reactions of acetates, chlorides citrates, sodium salts, potassium salts, calcium salts and magnesium salts (2.3.1).

Tests

pH (2.4.24). 3.0 to 7.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For calcium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm and using *calcium solution FP* or *calcium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For magnesium — To 50.0 ml add 50 ml of *water* and 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.005 M disodium edetate* using 50 mg of *eriochrome black T mixture* as indicator. Carry out a blank titration using 17.5 mg of $CaCl_2.2H_2O$ dissolved in 50 ml of distilled *water*, add 5.0 ml of *ammonia-ammonium chloride solution* and dilute to 250 ml with *water*. Calculate the content of magnesium with the volume obtained by subtracting the volume of EDTA required for calcium from the consumption of *0.005 M EDTA* in the titration of magnesium. Carry out a blank titration using 17.5 mg of $CaCl_2.2H_2O$ dissolved in 50 ml of distilled *water*, add 5.0 ml of *ammonia-ammonium chloride solution* and dilute to 250 ml with *water*. Calculate the content of magnesium with the volume obtained by subtracting the volume of EDTA required for calcium from the consumption of *0.005 M EDTA* in the titration of magnesium.

1 ml of *0.005 M disodium edetate* is equivalent to 0.1215 mg of Mg.

For acetate — Determine by liquid chromatography (2.4.14).

Test solution. Dilute a measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of Sodium Acetate.

Reference solution. Dissolve a weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution. Calculate the content of acetate in the preparation under examination.

For citrate — Determine by liquid chromatography (2.4.14).

Test solution. Preparation under examination.

Reference solution. Dissolve a weighed quantity of anhydrous *sodium citrate*, previously dried at 180° for 18 hours, in *water* to obtain a stock solution having a known concentration of about 10 mg per ml. Dilute measured volumes of the solution quantitatively with *water* to obtain three solutions having known concentrations of about 0.5, 1.0 and 2.0 mg, respectively of anhydrous sodium citrate per ml.

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution containing 1.0 mg of anhydrous sodium citrate per ml. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and all the three preparations of reference solution and measure the responses for the major peak. Plot the responses of all the three preparations of reference solution versus concentration, in mg of anhydrous sodium citrate per ml, and draw the straight line best fitting the three plotted points. From the graph so obtained, calculate the content of citrate in mg equivalent to anhydrous sodium citrate per litre of the preparation under examination.

For total chloride — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose —To a measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$ in the volume taken for assay.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

Multiple Electrolytes Injection Type VI

Multiple Electrolytes Injection Type VI is a sterile solution of suitable salts in Water for Injections to provide sodium, potassium, calcium, magnesium, acetate, citrate and chloride ions. It may contain Hydrochloric Acid or Sodium Hydroxide used for adjusting the pH.

Usual strength.

Sodium acetate	0.64 g
Sodium chloride	0.5 g
Sodium citrate	0.075 g
Potassium chloride	0.075 g
Calcium chloride	0.035 g
Magnesium chloride	0.031 g
Water for Injections to	100 ml
Concentration of electrolytes in mmol / l	
Sodium	140.0
Potassium	10.0
Calcium	2.5
Magnesium	1.5
Acetate	47.0
Chloride	103.0
Citrate	2.5

Multiple Electrolytes Injection Type VI contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, calcium, Ca, magnesium, Mg, acetate, $C_2H_3O_2$, and citrate, $C_6H_5O_7$. It also contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of chloride, Cl. It contains no antimicrobial agent.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

20 ml gives the reactions of acetates, chlorides citrates, sodium salts, potassium salts, calcium salts and magnesium salts (2.3.1).

Tests

pH (2.4.24). 3.0 to 7.0.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *For total sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For calcium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm and using *calcium solution FP* or *calcium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For magnesium — To 50.0 ml add 50 ml of *water* and 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.005 M disodium edetate* using 50 mg of *eriochrome black T mixture* as indicator.

1 ml of *0.005 M disodium edetate* is equivalent to 0.1215 mg of Mg.

For acetate — Determine by liquid chromatography (2.4.14).

Test solution. Dilute a measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 0.12 per cent w/v of *Sodium Acetate*.

Reference solution. Dissolve a weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of sodium acetate.

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent. Inject the reference solution and the test solution. Calculate the content of acetate in the preparation under examination.

For citrate — Determine by liquid chromatography (2.4.14).

Test solution. Preparation under examination.

Reference solution. Dissolve a weighed quantity of anhydrous *sodium citrate*, previously dried at 180° for 18 hours, in *water* to obtain a stock solution having a known concentration of about 10 mg per ml. Dilute measured volumes of the solution quantitatively with *water* to obtain three solutions having known concentrations of about 0.5, 1.0 and 2.0 mg, respectively of anhydrous sodium citrate per ml.

Chromatographic system

- a stainless steel column 30 cm × 7.8 mm, packed with strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 mm) and a guard column 4 cm × 7.8 mm packed with the same column material,
- column. temperature 60°,
- mobile phase: *0.1 M sulphuric acid,*
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 20 µl.

Inject the reference solution containing 1.0 mg of anhydrous *sodium citrate* per ml. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and all the three preparations of reference solution and measure the responses for the major peak. Plot the responses of all the three preparations of reference solution versus concentration, in mg of anhydrous sodium citrate per ml, and draw the straight line best fitting the three plotted points. From the graph so obtained, calculate the content of citrate in mg equivalent to anhydrous sodium citrate per litre of the preparation under examination.

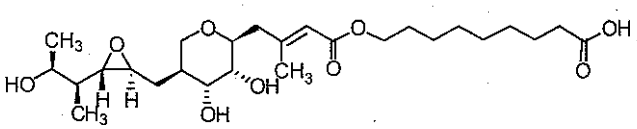
For total chloride — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

Storage. Store in single dose containers at a temperature not exceeding 30°.

Labelling. The label states (1) the content of each electrolyte in terms of millimoles in a given volume; (2) the amount of each ingredient in 100 ml; (3) the total osmolar concentration in mOsmol per litre; (4) that the preparation should not be used if it contains visible particles.

Mupirocin



$C_{26}H_{44}O_9$ Mol. Wt. 500.6

Mupirocin is 9-[(*E*)-4-[(2*S*,3*R*,4*R*,5*S*)-3,4-Dihydroxy-5-[[(2*S*,3*S*)-3-[(2*S*,3*S*)-3-hydroxy-2-butanyl]-2-oxiranyl]methyl]-2-oxanyl]-3-methyl-2-butenoyl]oxynonanoic acid.

Mupirocin is produced by the growth of certain strains of *Pseudomonas fluorescens*or obtained by any other means.

Mupirocin contains not less than 93.0 per cent and not more than 102.0 per centof $C_{26}H_{44}O_9$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or almost white powder. It shows polymorphism (2.5.11).

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mupirocin IPRS* or with the reference spectrum of mupirocin.

Tests

pH(2.4.24). 3.5 to 4.0 for a freshly prepared saturated solution in *carbon dioxide- free water.*

Specific optical rotation (2.4.22). – 21.0° to – 17.0°, determined in 5.0 per cent w/v solution in *methanol.*

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Mix 50 volumes of *methanol* and 50 volumes of a 1.36 per cent w/v solution of *sodium acetate*, adjusted to pH 4.0 with *acetic acid.*

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution(a). Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture.

Reference solution (b). Adjust 10 ml of reference solution (a) to pH 2.0 with *hydrochloric acid* and allow to stand for 20 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *water*, 30 volumes of *tetrahydrofuran* and 50 volumes of a 1.05 per cent w/v solution of *ammonium acetate*, adjusted to pH 5.7 with *acetic acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

The relative retention time with respect to mupirocin for pseudomonic acid D (mupirocin impurity C) is about 0.75.

Inject reference solution (b). This test is not valid unless resolution between the second of the 2 peaks due to hydrolysis products and the peak due to mupirocin is not less than 7.0 in the chromatogram obtained with reference solution (b).

Inject reference solution (a) and the test solution. Run the chromatogram 3.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to mupirocin impurity C is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (6.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 5 ml of *methanol* and dilute to 200.0 ml with a 0.75 per cent w/v solution of *ammonium acetate*, adjusted to pH 5.7 with *acetic acid.*

Reference solution(a). Dissolve 25 mg of *mupirocin lithium IPRS* in 5 ml of *methanol* and dilute to 200.0 ml with a0. 75 per cent w/v solution of *ammonium acetate*, adjusted to pH 5.7 with *acetic acid.*

Reference solution (b). Adjust 10 ml of the test solution to pH 2.0 with *hydrochloric acid* and allow to stand for 20 hours.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 19 volumes of *water*, 32 volumes of *tetrahydrofuran* and 49 volumes of a 1.05 per cent *ammonium acetate solution*, adjusted to pH 5.7 with *acetic acid.*
- flow rate: 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

Inject reference solution (a) and (b). This test is not valid unless resolution between the second of the 2 peaks due to hydrolysis products and the peak due to mupirocin is not less than 7.0 in the chromatogram obtained with reference solution (b). The relative standard deviation for replicate injections is not more than 2.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the contentof $C_{26}H_{44}O_9$.

1 mg of mupirocin lithium is equivalent to 0.988 mg of mupirocin.

Storage. Store protected from light.

Mupirocin Ointment

Mupirocin Ointment contains Mupirocin in a suitable base.

Mupirocin Ointment contains not less than 90.0 per cent and not more than 115.0 per cent ofthe stated amount of $C_{26}H_{44}O_9$.

Usual strength. 2 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 2 volumes of *glacial acetic acid*, 5 volumes of *methanol* and 93 volumes of *ethyl acetate.*

Test solution. Disperse a quantity of the ointment containing 20 mg of Mupirocin in 20 ml of *methanol*, with the aid of ultrasound for 20 minutes and filter.

Reference solution (a). A 0.1 per cent w/v solution of *mupirocin lithium IPRS* in *methanol.*

Reference solution (b). Add *0.2 M hydrochloric acid*, dropwise, to 10 ml of reference solution (a), to adjust pH 2.0 and allow to stand at room temperature for 20 hours (generation of hydrolysis products).

Apply to the plate 25 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots at lower R_f values than the spot due to mupirocin.

B. In the Assay, the principal peak in the chromatogram obtained withthe test solution corresponds to that of the principal peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the ointment containing 50 mg of Mupirocin in 50 mlof *0.1 Mphosphate buffer solution pH 6.3*, swirl and allow to stand (with occasional swirling) until dissolution is almost complete. Shake vigorously for 10 minutes and add sufficient *0.1 Mphosphate buffer solution pH 6.3* to produce 100 ml.

Reference solution. A 0.00054 per cent w/v solution of *mupirocin lithium IPRS* in *0.1 M phosphate buffer solution pH 6.3.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (7 µm) (Such as Zorbax C8),
- mobile phase: a mixture of 28 volumes of *tetrahydrofuran* and 72 volumes of a buffer solution prepared by dissolving 7.7 g of *ammonium acetate* in 900 ml of *water*, adjusted to pH 5.7 with *0.1 M glacial acetic acid* and diluting to 1000 ml with *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume: 20 µl.

Name	Relative retention time
Mupirocin impurity D[1]	0.5
Mupirocin impurity E[2]	0.55
Mupirocin impurity C[3]	0.65

[1] 9-[[(2*E*)-4-[(2*R*,3a*S*,6*S*,7*S*)-2-[(2*S*,3*S*)-1,3-dihydroxy-2-methylbutyl]-7-hydroxyhexahydro-4*H*-furo[3,2-*c*]pyran-6-yl]-3-methylbut-2-enoyl]oxy]nonanoic acid,

[2] 9-[[(2*E*)-4-[(2*R*,3*RS*,4a*S*,7*S*,8*S*,8a*R*)-3,8-dihydroxy-2-[(1*S*,2*S*)-2-hydroxy-1- methylpropyl]hexahydro-2*H*,5*H*-pyrano[4,3-*b*]pyran-7-yl]-3-methylbut-2- enoyl]oxy]nonanoic acid,

[3] pseudomonic acid D.

Inject the reference solution. This test is not valid unless resolution between the peaks corresponding to mupirocin and the peak with a relative retention time of about 0.65 (mupirocin impurity C) is not less than 3.5.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to mupirocin impurity C is not more than four times the area of the principal peak in the chromatogram obtained with the reference solution (4.0 per cent). The area of any peak corresponding to mupirocin impurity D is not more than 5 times the area of the principal peak in the chromatogram obtained withthe reference solution (5.0 per cent). The area of any peak corresponding to mupirocin impurity E is not more than 10 times the area of the principal peak in the

chromatogram obtained with the reference solution (10.0 per cent), the area of any other secondary peak is not more than 1.5 times the area of the principal peak in the chromatogram obtained withthe reference solution (1.5 per cent)and the sum of areas of all the secondary peaks is not more than 20 times the area of the principal peak in the chromatogram obtained withthe reference solution (20.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent).

Other tests. Comply with the tests stated under Ointment.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the ointment containing 50 mg of Mupirocin in 100 ml of *0.1 M phosphate buffer solution pH 6.3*, swirl and allow to stand (with occasional swirling) until dissolution is complete. Add 56 ml of *acetonitrile* and sufficient *0.1 M phosphate buffer solution pH 6.3* to produce 200 ml.

Reference solution. A 0.027 per cent w/v solution of *mupirocin lithium IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as µBondapack C18),
- mobile phase: a mixture of 28 volumes of *acetonitrile* and 72 volumes of *0.1 M phosphate buffer solution pH 6.3*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume: 20 µl.

The retention time of mupirocin is about 5 minutes. The relative retention time of mupirocin impurity C to mupirocin is about 0.77.

Inject the reference solution. This test is not valid unless resolution between the peaks corresponding to mupirocin and the peak corresponding to mupirocin impurity C is not less than 1.5.

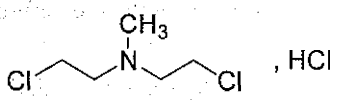
Inject the reference solutionand the test solution.

Calculate the content of $C_{26}H_{44}O_9$ in the ointment.

1 mg of $C_{26}H_{43}O_9Li$ is equivalent to 0.9882 mg of $C_{26}H_{44}O_9$.

Mustine Hydrochloride

Nitrogen Mustard



$C_5H_{11}Cl_2N,HCl$ Mol Wt. 192.4

Mustine Hydrochloride is bis(2-chloroethyl)methylamine hydrochloride.

Mustine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_5H_{11}Cl_2N$, HCl.

Category. Anticancer.

Description. A white or almost white, crystalline powder or mass; hydroscopic; vesicant.

Identification

A. Dissolve 50 mg in 5 ml of *water* and add 1 ml of *5 M sodium hydroxide*; oily globules are produced which dissolve on warming.

B. Dissolve 50 mg in 5 ml of *water* and add 0.02 ml of *potassium mercuri-iodide solution*; a cream-coloured precipitate is produced.

A. Melts at about 108° (2.4.21).

Tests

Assay. Weigh 0.2 g, add 15 ml of *1 M ethanolic potassium hydroxide* and 15 ml of *water* and boil under a reflux condenser for 2 hours. Evaporate the solution to half its volume on water-bath, dilute to 150 ml with *water*, add 3 ml of *nitric acid* and 50.0 ml of *0.1 M silver nitrate*. Shake vigorously and filter. Wash the residue with *water* and titrate the excess of silver nitrate in the combined filtrate and washings with *0.1 M ammonium thiocycanate* using 1 ml of *ferric ammonium sulphate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.006418 g of $C_5H_{11}Cl_2N$, HCl.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states that the contents of the container are strongly vesicant.

Mustine Injection

Mustine Hydrochloride Injection

Mustine Injection is a sterile material consisting of Mustine Hydrochloride with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections or Sodium Chloride Intravenous Infusion, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution deteriorates rapidly on storage and should be used immediately after preparation.

Mustine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of the stated amount of mustine hydrochloride, $C_5H_{11}Cl_2N,HCl$.

Usual strength. 10 mg.

The contents of the sealed container complies with the tests stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

Dissolve about 20 mg in 1 ml of *water* and add 0.02 ml of *potassium mercuri-iodide solution*; a cream-coloured precipitate is produced.

Tests

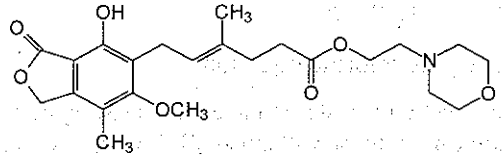
Assay. Determine the weight of the contents of 10 containers. Weigh a quantity of the mixed contents of the ten containers, containing 40 mg of Mustine Hydrochloride, dissolve in 10 ml of *ethanol (95 per cent)*, previously neutralised to dilute *phenolphthalein solution*. Titrate with *0.01 M sodium hydroxide* using *dilute phenolphthalein solution* as indicator.

1 ml of *0.01 M sodium hydroxide* is equivalent to 0.001925 g of $C_5H_{11}Cl_2N,HCl$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) that the contents are strongly vesicant; (2) the amount of Mustine Hydrochloride in the container, (3) that the injection should be used immediately after preparation.

Mycophenolate Mofetil



$C_{23}H_{31}NO_7$ Mol. Wt. 433.5

Mycophenolate Mofetil is 2-(morpholin-4-yl)ethyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

Mycophenolate Mofetil contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{23}H_{31}NO_7$, calculated on the dried basis.

Category. Immunosupressant.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *mycophenolate mofetil IPRS* or with the reference spectrum of mycophenolate mofetil.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use. Protect the solutions from light.

Test solution. Dissolve 200 mg of the substance under examination in 100.0 ml of *acetonitrile.*

Reference solution. A 0.001 per cent w/v solution of *mycophenolate mofetil IPRS* in *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (such as Zorbax SB-C8),
- column temperature: 45°,
- sample temperature. 10°,
- mobile phase: a mixture 65 volumes of *water*, 0.2 volumes of *triethylamine*, adjusted to pH 5.3 with *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent. The relative retention time with reference to the principal peak for impurity F is about 0.3, for impurity A is about 0.4, for impurity H is about 0.5, for impurity G is about 0.6, for impurity B is about 0.8, for impurity D is about 1.2 and for impurity E is about 1.6.

Inject the reference solution and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of peak due to impurity A, B, D, E, F, G, H or any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying it at 60° for 3 hours under vacuum.

Assay. Weigh 0.4 g and dissolve in 50 ml of *glacial acetic acid* and titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04335 g of $C_{23}H_{31}NO_7$.

Storage. Store protected from light and moisture.

Mycophenolate Mofetil Capsules

Mycophenolate Mofetil Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of mycophenolate mofetil, $C_{23}H_{31}NO_7$.

Usual strengths. 250 mg; 500 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.0025 per cent w/v solution in *methanol* shows an absorption maximum as obtained with *mycophenolate mofetil IPRS* of the same concentration.

Tests

Dissolution (2.5.2).

Apparatus No. 1 (Basket),
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. Dissolve a quantity of *mycophenolate mofetil IPRS* in *methanol* and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.

Chromatographic system as described in the Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{31}NO_7$ in the capsule.

Q. Not less than 75 per cent of the stated amount of $C_{23}H_{31}NO_7$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Test solution. Mix the contents of 20 capsules. Disperse a quantity of the mixed content containing about 200 mg of Mycophenolate with *acetonitrile,* sonicate for 15 minutes and dilute to 200.0 ml with *acetonitrile.*

Reference solution. A 0.001 per cent w/v solution of *mycophenolate mofetil IPRS* in *acetonitrile.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of buffer solution prepared by dissolving 13.6 g of *potassium dihydrogen orthophosphate anhydrous* in 1000 ml of *water*, adjusted to pH 3.6 with *orthophosphoric acid* and 4.0 g of *sodium heptane sulphonate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent); the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (2.0 per cent).

Water (2.3.43). Not more than 4.0 per cent, determined on 0.5 g.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

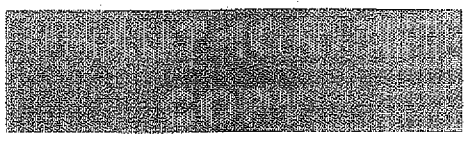
NOTE—Use freshly prepared solutions.

Test solution. Disperse a quantity of the content of the capsules containing about 500 mg of Mycophenolate Mofetil with *methanol*, sonicate for 15 minutes and dilute to 500.0 ml with the *methanol*. Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase.

Reference solution. Dissolve 10 mg of *mycophenolate mofetil IPRS* in sufficient amount of *methanol*, sonicate for 15 minutes and dilute to 20.0 ml with *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3V),



- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of buffer solution prepared by dissolving 13.6 g of *potassium dihydrogen orthophosphate anhydrous* in 1000 ml with *water*, adjusted to pH 3.6 with *orthophosphoric acid* and dissolve 4.0 g of *sodium heptane sulphonate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume: 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000 and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference sollution and the test solution.

Calculate the content of $C_{23}H_{31}NO_7$ in the capsules.

Storage. Store protected from light, at a temperature not exceeding 30°.

Mycophenolate Mofetil Oral Suspension

Mycophenolate Mofetil Oral Suspension is a dry mixture consisting of mycophenolate mofetil with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of sealed container in the specified volume of *water* just before use.

Mycophenolate Mofetil Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of mycophenolate mofetil, $C_{23}H_{31}NO_7$.

Usual strength. Mycophenolate Mofetil 200 mg per ml.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

pH (2.4.24). 6.0 to 7.0.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use and protect from light.

Buffer solution (a). Prepared by diluting 10.0 ml of *triethylamine* in 950 ml of *water*, adjusted to pH 7.2 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Buffer solution (b). Prepared by diluting 10.0 ml of *triethylamine* in 950 ml of *water*, adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*.

Solvent mixture (a). 35 volumes of *acetonitrile*, 20 volumes of buffer solution (b) and 45 volumes of *water*.

Solvent mixture (b). 50 volumes of *acetonitrile*, 15 volumes of buffer (b) and 35 volumes of *water*.

Test solution. Mix the contents of not less than 4 constituted containers. Transfer a volume of the constituted suspension containing about 800 mg of Mycophenolate Mofetil to a 200-ml volumetric flask, diluted with the solvent mixture (b) to the volume and mix. Dilute 5.0 ml of the solution to 50.0 ml with solvent mixture (a), mix and filter.

Reference solution (a). A solution containing 0.4 per cent w/v solution of *mycophenolate mofetil IPRS* in solvent mixture (b).

Reference solution (b). A solution containing 0.04 per cent w/v of *mycophenolate mofetil IPRS* in solvent mixture (a) prepared from reference solution (a).

Reference solution (c). A solution containing 0.001 per cent w/v each of *mycophenolate mofetil impurity A IPRS* and *mycophenolate mofetil impurity B IPRS* in the solvent mixture (a).

Reference solution (d). A solution containing 0.00002 per cent w/v of *mycophenolate mofetil IPRS* in solvent mixture (a) prepared from reference solution (b).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with phenyl group bonded to porous silica (5 µm),
- column temperature 45°,
- sample temperature 5°,
- mobile phase: a mixture of 30 volumes of *acetonitrile* and 70 volumes of a solution prepared by mixing 4 volumes of buffer solution (a) and 9 volumes of *water*.
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 249 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Mycophenolic acid[1]	0.12	0.71
Sorbitol ester of mycophenolic acid[2]	0.24	1.29
Mycophenolate mofetil impurity A	0.40	—
Mycophenolate mofetil impurity B	0.46	—
Mycophenolate mofetil	1.0	—
Any individual unspecified impurity	—	1.0

[1](*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid,

[2]Sorbitol (*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-isobenzofuran-5-yl)-4-methylhex-4-enoate,

[3]2-morpholinoethyl(E)-6-(1,3-dihydro-4,6-dihydroxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate,

[4]*(RS)*-7-hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3*H*-isobenzofuranyl-1-one.

Inject reference solution (b), (c) and (d). The test is not valid unless the resolution between mycophenolate mofetil impurity A and mycophenolate mofetil impurity B is not less than 2.0, signal-to-noise ratio for the principal peak in the chromatogram obtained with reference solution (d) is not less than 10.0, the tailing factor for the peak due to mycophenolate mofetil in the chromatogram obtained with reference solution (b) is not more than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent with reference solution (b).

Inject the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak due to mycophenolic acid is not more than 3.3 per cent. The area of any peak due to sorbitol ester of mycophenolic acid is not more than 0.2 per cent. The area of any secondary peak is not more than 0.1 per cent and the sum of the areas of all such peaks is not more than 3.8 per cent calculated by area normalization. Ignore any peak with an area less than 0.05 per cent.

Other tests. Comply with the tests stated under Oral Powders.

Assay. Determine by liquid chromatography (2.4.14) as described under related substances using the following solutions.

Inject reference solution (b). The test is not valid unless the tailing factor of the principal peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b) and the test solution.

Calculate the content of $C_{23}H_{31}NO_7$ in the suspension.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{23}H_{31}NO_7$, weight in volume.

Repeat the procedure using a portion of the constituted suspension that has been stored at the temperature and for the period stated on the label.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) the quantity of active ingredient in terms of the equivalent amount of mycophenolate mofetil; (2) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

Mycophenolate Mofetil Tablets

Mycophenolate Mofetil Tablets contain not less than 94.0 per cent and not more than 105.0 per cent of the stated amount of mycophenolate mofetil, $C_{23}H_{31}NO_7$.

Usual strengths. 250 mg; 360 mg; 500 mg; 750 mg.

Identification

A. When examined in the range 200 nm to 400 nm (2.4.7), the solution obtained in the dissolution test shows an absorption maxima and minima at the same wavelength ±3 as that of reference solution of *mycophenolate mofetil* prepared in the same manner.

B. In the Assay, the chromatogram obtained with the test solution corresponds to the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm, 5 minutes and 15 minutes.

Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary with the dissolution medium. Measure the absorbance of the resulting solution at the maximum at about 250 nm using 1 cm path length (2.4.7). Calculate the content of $C_{23}H_{31}NO_7$ in the medium from the absorbance obtained from 0.0011 per cent w/v solution of *mycophenolate mofetil IPRS* in the dissolution medium and dilute the solution as that similar concentration of test solution in the same medium.

Calculate the content of $C_{23}H_{31}NO_7$ in the medium.

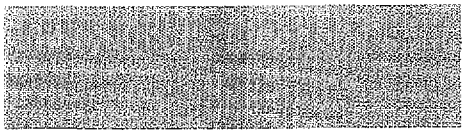
Q. Not less than 70 per cent in 5 minutes and not less than 85 per cent in 15 minutes, of the stated amount of $C_{23}H_{31}NO_7$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the powder tablets containing about 250 mg of *mycophenolate mofetil* with 10.0 ml of *water*, shake mechanically for a minimum of 15 minutes and add 70.0 ml of *acetonitrile*. Sonicate for 15 minutes, again shake mechanically for 20 minutes and make up to 100.0 ml with *acetonitrile*. Dilute 5.0 ml of the solution to 100.0 ml with the *acetonitrile* and filter.

Reference solution (a). A 0.0125 per cent w/v solution of *mycophenolate mofetil IPRS* in *acetonitrile*.

Reference solution (b). A 0.00000625 per cent w/v solution of *mycophenolate mofetil IPRS* in *acetonitrile*.



Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- sample temperature.10°,
- column temperature:45°,
- mobile phase: a mixture of 45 volumes of a buffer solution prepared by diluting 3.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 5.3 with *orthophosphoric acid* and 55 volumes of *acetonitrile*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
Mycophenolic acid[1]	0.6	0.71
Mycophenolate *N*-oxide analog[2]	0.8	1.0
Mycophenolate mofetil	1.0	—
Any single unspecified impurity	—	1.0

[1](*E*)-6-(1,3-Dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid,

[2]2-Morpholinoethyl (*E*)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate N-oxide.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 in the chromatogram obtained with reference solution (a) and and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject reference solution (b). The chromatogram obtained shows one principal peak with a signal-to-noise ratio of not less than 10.

Inject reference solution (a) and the test solution. Run the chromatogram 3.0 times the retention time of the principal peak in the chromatogram obtained with the test solution, the area of any peak corresponding to mycophenolic acid is not more than 0.01 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and any peak corresponding to mycophenolate *N*-oxide analog is not more than 0.002 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any secondary peak is not more than 0.001 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of area of all secondary peaks is not more than 0.015 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). Ignore any peak with a relative retention times of 1.45, and 2.15, any peak with an area less than 0.0005 times the area of the peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances with the following modifications.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 250 mg of Mycophenolate Mofetil with 10.0 ml of *water*, shake mechanically for a minimum of 15 minutes and add 70.0 ml of *acetonitrile*. Sonicate for 15 minutes, again shake mechanically for 20 minutes and make up to 100.0 ml with *acetonitrile*. Dilute 5.0 ml of the solution to 100.0 ml with the *acetonitrile* and filter.

Reference solution. A 0.0125 per cent solution of *mycophenolate mofetil IPRS* in *acetonitrile*.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{23}H_{31}NO_7$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Myristic Acid

$CH_3(CH_2)_{12}COOH$

$C_{14}H_{28}O_2$ Mol. Wt. 228.4

Myristic acid is tetradecanoic acid.

Myristic Acid is obtained from coconut oil and other fats.

Myristic Acid contains not less than 97.0 per cent of $C_{14}H_{28}O_2$.

Category. Pharmaceutical aid.

Tests

Congealing temperature (2.4.10). 48° to 55.5°.

Acid value (2.3.23). 242 to 249.

Saponification value (2.3.37). 242 to 251.

Iodine value (2.3.28). Not more than 1.0.

Unsaponifiable matter (2.3.39). Not more than 1.0 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.2 per cent.

Lead. Determine by atomic absorption spectrophotometry (2.4.2), measuring at 283.3 nm using a lead electrodeless discharge lamp, an air acetylene flame, and a suitable burner head, preferably a slit-width of 0.7 nm.

NOTE—Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 8 M nitric acid followed by deionized water.

Test solution. Weigh 5 g of Myristic Acid to an evaporating dish, add 5 ml of a 25 per cent *sulphuric acid solution* and distribute the *sulphuric acid* uniformly through the sample. Within a hood, place the dish on a steam bath to evaporate most of the *water*. Place the dish on a burner, and slowly pre-ash the sample by expelling most of the *sulphuric acid*. Place the dish in a muffle furnace that has been set at 525°, and ash the sample until the residue appears free from carbon. Prepare a blank by ashing 5 ml of 10 per cent *sulphuric acid solution*. Cool, and cautiously wash down the inside of each evaporation dish with water. Treat both the sample and the blank as follows. Add 5 ml of *1 M hydrochloric acid*. Place each dish on a steam bath, and evaporate to dryness. To each dish add 1.0 ml of *3 M hydrochloric acid* and approximately 5 ml of *water*, and heat briefly on a steam bath to dissolve any residue. Transfer each solution quantitatively to a 10-ml volumetric flask, dilute with *water* to volume, and mix.

Reference solution (a). Weigh 0.16 g of *lead nitrate* in 1 per cent v/v solution of *nitric acid*. Dilute to 1000 ml with *water* and mix.

Reference solution (b). Dissolve 10.0 ml of reference solution (a) in *water* to a 100-ml volumetric flask and mix. 1 ml of the solution contains the equivalent of about 10 µg of lead. Dilute measured volumes of the diluted reference solution (a) with *water* to obtain solutions having known concentrations of about 1 µg, 2 µg, and 5 µg of lead per ml.

NOTE—Prepare these solutions on the day of use.

Calculate the content of lead, in the substance under examination. (2 ppm).

Assay. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 100 mg of the substance to a 50-ml conical flask fitted with a suitable water-cooled reflux condenser and a magnetic stir bar. Add 4 ml of *0.5 M sodium hydroxide solution methanolic*, and reflux until fat globules disappear (usually 5 to 10 minutes). Add 5 ml of a solution prepared by dissolving 14 g of boron trifluoride in *methanol* to make 100 ml, swirl to mix, and reflux for 2 minutes. Add 4 ml of chromatographic *n-heptane* through the condenser, and reflux for 1 minute. Cool, remove the condenser, add about 15 ml of *saturated sodium chloride solution*, shake, and allow the layers to separate. Pass the n-heptane layer through 0.1 g of *anhydrous sodium sulphate* (previously washed with chromatographic *n-heptane*) into a suitable flask. Transfer 1.0 ml of the solution to a 10-ml volumetric flask, dilute with chromatographic *n-heptane* to volume, and mix.

Reference solution (a). Prepare as directed for the test solution using 100 mg of *myristic acid IPRS* instead of the substance under examination.

Reference solution (b). Dissolve about 20 mg each of stearic acid, palmitic acid and oleic acid to a 25-ml conical flask fitted with a suitable water-cooled reflux condenser and a magnetic stir bar, and proceed as directed for the test solution, beginning with "Add 5.0 ml of a solution prepared by dissolving."

Chromatographic system

- a fused-silica capillary column 30 m x 0.53 mm, packed with bonded with a 1.0 µm layer of phase G16,
- temperature:
  column.70° for 2 minutes and 240° for 5 minutes,
  inlet port. 220°,
- a flame ionisation detector,
- flow rate: 30 ml per minute using nitrogen as the carrier gas.

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to methyl stearate and methyl palmitate is not less than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject 1 µl of reference solution (a) and the test solution.

Calculate the content $C_{14}H_{28}O_2$ by area normalization method.

Storage. Store protected from light and moisture.